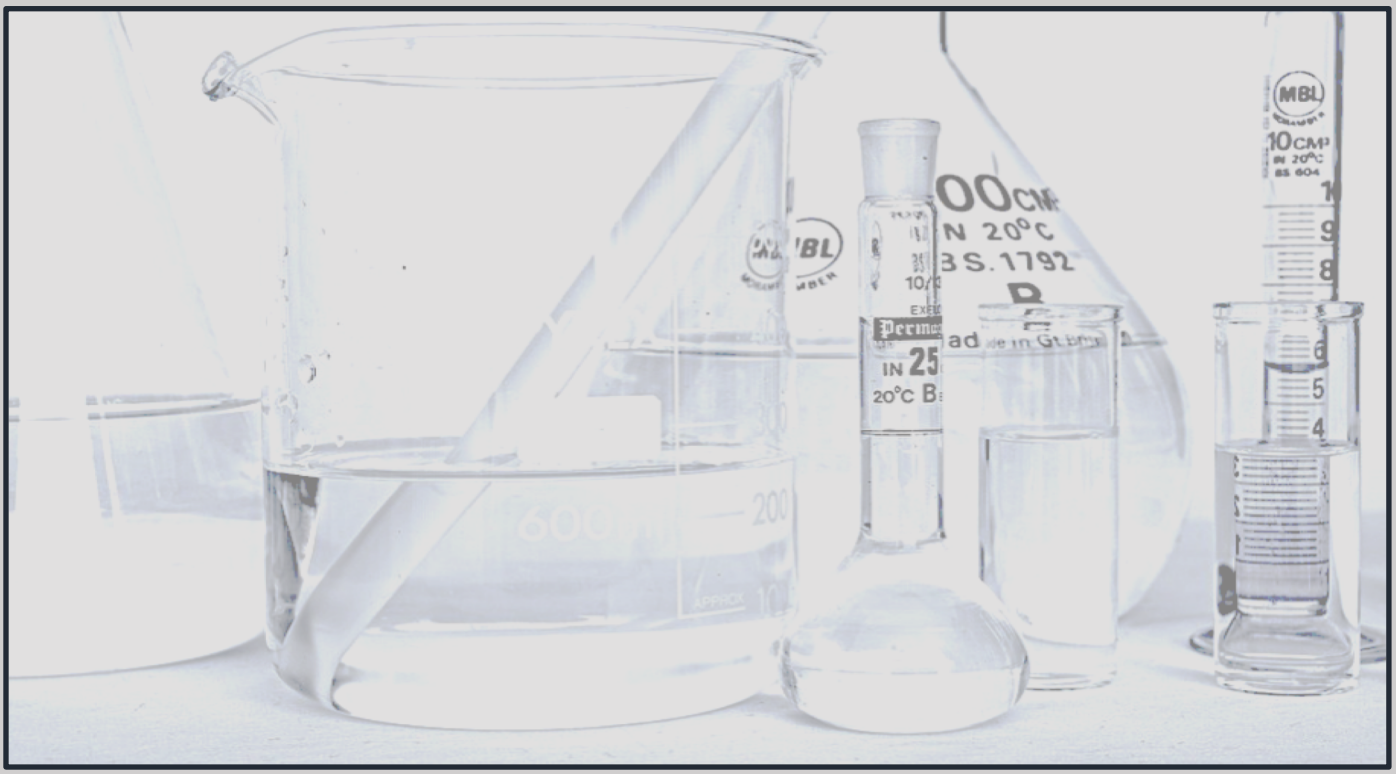
2nd Annual ADME: Lead Characterization and Optimization Conference

Oct. 22, 2020



Gulf Coast Consortia

QUANTITATIVE BIOMEDICAL SCIENCES

THE STATE OF TEXAS

CPRIT

Gulf Coast Consortia

QUANTITATIVE BIOMEDICAL SCIENCES

The Gulf Coast Consortia (GCC), located in Houston, Texas, is a dynamic, multi-institution collaboration of basic and translational scientists, researchers, clinicians and students in the quantitative biomedical sciences, who benefit from joint training programs, topic-focused research consortia, shared facilities and equipment, and exchange of scientific knowledge. Working together, GCC member institutions provide a cutting-edge collaborative training environment and research infrastructure beyond the capability of any single institution. GCC training programs currently focus on Biomedical Informatics, Computational Cancer Biology, Molecular Biophysics, Pharmacological Sciences, Precision Environmental Health Sciences and Antimicrobial Resistance. GCC research consortia gather interested faculty around research foci within the quantitative biomedical sciences, and currently include Antimicrobial Resistance, Cellular and Molecular Biophysics, Innovative Drug Discovery and Development, Immunology, Mental Health, Regenerative Medicine, Single Cell Omics, Theoretical and Computational Neuroscience, Translational Imaging and Translational Pain Research. Current members include Baylor College of Medicine, Rice University, University of Houston, The University of Texas Health Science Center at Houston, The University of Texas Medical Branch at Galveston, The University of Texas M. D. Anderson Cancer Center, and the Institute of Biosciences and Technology of Texas A&M Health Science Center.

Gulfcoastconsortia.org

GCC CENTER FOR COMPREHENSIVE

PK/PD & FORMULATION

The Gulf Coast Consortia (GCC) Center for Comprehensive PK/PD & Formulation (CCPF), located at Texas Southern University, is CPRIT-funded state-of-the-art drug development core facility with faculty experts from Texas Southern University, University of Houston College of Pharmacy, and the GCC for Quantitative Biomedical Science. Primary focus of CCPF is on preclinical drug development to facilitate rapid advancement of novel cancer drugs from bench to clinical trials. Specifically CCPF core provides service in nine preclinical drug development aspects: (1) drug pre-formulation characterizations; (2) pharmaceutical dosage form development; (3) advanced drug delivery systems; (4) analytical LC-MS/MS method development and validation; (5) in vitro drug metabolism; (6) in vitro permeability and protein binding; (7) pharmacokinetic (PK) studies; (8) in vitro and in vivo pharmacodynamics (PD); and (9) PK/PD modeling and simulation studies. CCPF core laboratories are equipped with major instruments such as UPLC-PDA and LC-MS/MS for bioanalysis, accurate mass spectrometer for unknown metabolite identification, USP II dissolution apparatus and smart flow-through dissolution system for drug release studies, Pion Sirius T3 automated system for drug physicochemical profiles, and Malvern Nano Zetasizer for nanoparticles. Rodent animal studies are conducted at the animal facility within the Laboratory Animal Resources of Texas Southern University. CCPF core is also equipped with computers and software at University of Houston required for advance PK/PD modeling.

gcc-ccpf.com

Agenda

10:00 *Welcome and Introduction to GCC Center for Comprehensive PK/PD and Formulation (CCPF)*
Suzanne Tomlinson, PhD, Gulf Coast Consortia
Huan Xie, PhD, Texas Southern University
Dong Liang, PhD, Texas Southern University
Diana Chow, PhD, University of Houston
Omonike Olaleye, PhD, MPH, Texas Southern University

10:10 **Keynote:** *Chemo-immunotherapy for Colorectal Cancer and Liver Metastasis*
Leaf Huang, PhD
University of North Carolina
(Introduction by Dr. Chow)

Session 1: Formulation and PK
Convener: Huan Xie, PhD, CCPF-CPRIT Core PD

10:50 *Supralingual Delivery of Mycophenolic Acid – PK Analysis and Modeling*
Robert YL Tsai, MD, PhD
Texas A&M Institute of Biosciences and Technology
Xiaohua Liu, PhD
Texas A&M College of Dentistry
Huan Xie, PhD
Texas Southern University

11:15 *Targeting Skp2/Ck1 to Restore Nuclear p27 in Cancers with Mislocalized p27*
Ruhee Dere, PhD
Baylor College of Medicine

11:35 *Trends of Oral Formulation in Cancer Care: Focus on Capecitabine*
Veronica Ajewole, PharmD, BCOP
Texas Southern University

11:55 *Overview of the PD Facility of the CCPF*
Yun Zhang, PhD
Texas Southern University

12:15 **Lunch Break and Virtual Poster session**
12:15-12:55 Poster Session

1:30 **Keynote:** *Model-Informed Drug Development: Challenges and Opportunities*
Stephan Schmidt, PhD
University of Florida
(Introduction by Dr. Chow)

Session 2: PK/PD and Metabolism
Convener: Dong Liang, PhD, CCPF-CPRIT PI

2:10 *Characterization and Optimization of Thyroid Hormone Metabolites for Type 1 Diabetes Treatment*

Carly Filqueira, PhD
Houston Methodist Research Institute
Dong Liang, PhD
Texas Southern University

2:30 *The Multiple Activities of Jumonji Inhibitor JIB-04*
Elisabeth Martinez, PhD
UT Southwestern Medical Center

2:50 *Alleviating Irinotecan-induced Diarrhea with Herbal Mixture Xiao-Chai-Hu-Tang (XCHT)*
Song Gao, PhD
Texas Southern University

3:10 Wrap up followed by virtual networking

ADME Steering Committee:

Diana Chow, University of Houston
Dong Liang, Texas Southern University
Omonike Olaleye, Texas Southern University
Suzanne Tomlinson, Gulf Coast Consortia
Huan Xie, Texas Southern University

Speaker abstracts in order of appearance

Leaf Huang, PhD
Fred Eshelman Distinguished Professor
Division of Pharmacoengineering and Molecular Pharmaceutics

Chemo-immunotherapy for Colorectal Cancer and Liver Metastasis

Leaf Huang, Ph.D. is the Fred Eshelman Distinguished Professor, Division of Pharmacoengineering and Molecular Pharmaceutics in the Eshelman School of Pharmacy, University of North Carolina at Chapel Hill. Dr. Huang's research has been in gene therapy and targeted drug delivery. He has pioneered the liposome non-viral vector and has designed and manufactured the cationic lipid vector for the first non-viral clinical trial in 1992. His current work centers on nanoparticle vectors for gene transfer in tumor and liver. He also continues research in establishing a ligand targeted delivery system for cDNA, mRNA, siRNA, proteins and peptides for remodeling the tumor microenvironment, leading to tumor growth inhibition. He has authored or co-authored more than 600 papers with an H-index of 126. He is also the inventor or co-inventor of 22 US and foreign patents. In 2004, he received the Alec D. Bangham MD FRS Achievement Award, which is the highest honor in liposome research. He was the recipient of the 2013 Distinguished Pharmaceutical Scientist Award which is the highest scientific recognition of the American Association of Pharmaceutical Scientists. He was named a Highly Cited Researcher in "Pharmacology & Toxicology" since 2016. Dr. Huang has also co-founded 6 biotech start-ups in the past.

Abstract: Colorectal cancer accounts for about 10% of cancer death. Most of the CRC are resistant to checkpoint inhibitor therapy. We have used nanotechnology to deliver drugs and genes to remodel the tumor microenvironment to facilitate immunotherapy for the primary tumor and the liver metastasis. Several different strategies will be discussed in the talk. The work is supported by NIH grant CA198999.

Robert Y. Tsai, MD, PhD
Associate Professor
Institute of Biosciences and Technology

Supralingual Delivery of Mycophenlic Acid: Pharmacokinetic Analysis and Modeling

Dr. Tsai received his M.D. degree from the National Taiwan University in 1988 and Ph.D. degree in Neuroscience from the Johns Hopkins University School of Medicine in 1996. He finished his Neurology residency training at The National Taiwan University Hospital in 1991 and Post-Doctoral training at National Institutes of Health in 2003. He is currently an Associate Professor at the Texas A&M University Health Science Center, Institute of Biosciences and Technology and has been a faculty member at Texas A&M since 2003. Dr. Tsai's research focuses on the discovery of new mechanisms driving stem cell self-renewal and the translation of this knowledge to medical applications that advance the management of tissue repair, premature aging, and tumor malignancy. His current research topics include: (1) delineating the molecular and cellular mechanism of nucleostemin that drives the self-renewal of normal and cancerous stem cells, with a special emphasis on how nucleostemin protects the integrity of genome against replication stress; (2) determining the roles of chromatin conformation and DNA methylation in liver regeneration, liver cancer progression, and liver aging; and (3) developing new screening models and novel therapeutic devices to predict and prevent cancer formation, with a special emphasis on oral squamous cell carcinoma and non-alcoholic fatty liver disease-related hepatocellular carcinoma. His work is funded by CPRIT and NIH and has led to a US patent and the establishment of a startup company, Post Oak Pharmaceuticals. Dr. Tsai has co-authored 45 peer-reviewed journal articles, 31 of which were published while he is at Texas A&M.

Abstract: Oral cancer is the 8th most common cancer in male and 15th in female in the US with a five-year survival rate remains at an abysmal 64% over decades. Those who survive oral cancer often have to live with impaired functions and

sometimes disfiguration caused by the treatment. Pathologically, more than 90% of the oral cancers are oral squamous cell carcinomas (OSCC) that may develop from clinically visible oral premalignant lesions (OPLs), which affect as many as 1.5-4.3% of the world population. A significant minority of OPLs (4-11% for low-grade dysplasia and 20-43% for high-grade dysplasia) can develop into OSCC. Currently, there is no suitable treatment for patients with high-grade OPLs, especially for those that are diffuse or multiple. We have discovered synergistically acting drug combinations, consisting of a chemo-sensitizing agent, mycophenolic acid (MPA) or mycophenolate (MPS), and various chemotherapeutic agents, that kill dysplastic oral keratinocytes as well as OSCC cells in culture. The goal of this study is to develop a new mucoadhesive drug-delivery device for MPA as a non-invasive option for treating OPLs. A computer-aided design (CAD) three-dimensional (3D) printing technique was used to formulate a novel mucoadhesive patch for topical delivery of MPS. The MPS patch product was tested for its in vitro and in vivo mucoadhesiveness, drug stability, and in vivo drug release profile in the mucosal tissue and plasma using different PK models. This study reports novel enterohepatic recycling (EHR) models for intravenous, oral and supralingual PK analyses. Our results show that the plasma concentration of MPA following supralingual administration is much lower compared to that following intravenous or oral administration, and that a large amount of MPA accumulates in the tongue tissue after patch application. The data suggest that MPA in the patch formulation is sustain-released from dorsal tongue.

Key word: oral cancer, oral dysplasia, mucoadhesive patch, 3-D printing, pharmacokinetic modeling

Xiaohua Liu, PhD
Associate Professor
Biomedical Sciences

Supralingual Delivery of Mycophenolic Acid – PK Analysis and Modeling

Dr. Xiaohua Liu is an Associate Professor at Texas A&M University College of Dentistry (TAMU-COD). He joined the Biomedical Sciences Department of TAMU-COD in 2010 and was promoted to tenured Associate Professor in 2016. Dr Liu's research focuses on cell-material interaction, biomimetic materials for craniofacial tissue regeneration, and controlled drug delivery. He is a member of the Society For Biomaterials, Biomedical Engineering Society, Materials Research Society, and International Association for Dental Research/American Association for Dental Research (IADR/AADR). He is an editorial board member of Journal of Dental Research, the best journal in the field of dentistry. He has published over 60 peer-reviewed papers on prestigious journals, including Nature Materials, Biomaterials, and Tissue Engineering, and his publications have been cited by other groups for more than 5800 times during the last 8 years. Dr. Liu's research is currently funded by two NIH grants. Dr. Liu received Basic Science Faculty Research Award (TAMU-COD) in 2018 and was selected as TAMU Presidential Impact Fellow in 2019.

Huan Xie, PhD
Pharmaceutics
College of Pharmacy and Health Sciences

Supralingual Delivery of Mycophenolic Acid – PK Analysis and Modeling

Dr. Huan Xie is Professor of Pharmaceutics at College of Pharmacy and Health Sciences, Texas Southern University. She received her B.S. in Chemistry from Fudan University in 1999 and Ph.D. in Analytical Chemistry from North Carolina State University in 2004. Dr. Xie has 4 years of industry experience contributing to the development of silica/gold nanoshell cancer therapy, now in several clinical trials. Since she joined TSU in 2008, she has been consistently funded by various agencies, and now serves as PI of Center of Biomedical and Minority Health Research (CBMHR) at TSU supported by NIH/NIMHD, and PD of GCC-Center of Comprehensive PK/PD and Formulation (CCPF) supported by CPRIT. She has a broad background and track record in nano-formulation and targeted drug delivery, with specific expertise in preclinical drug development. She is an inventor on 3 patents related to novel drug formulation and PK study and has many collaborators across Texas.

Ruhee Dere, PhD
Assistant Professor
Center for Precision Environmental Health

Targeting Skp2/Ck1 to Restore Nuclear p27 in Cancers with Mislocalized p27

I am currently an Assistant Professor in the Center for Precision Environmental Health at Baylor College of Medicine. I received my Ph.D. from Texas A&M University at the Institute of Biosciences and Technology in Houston. It was as a postdoctoral fellow, with Dr. Cheryl Walker, a leading expert in the field of renal cystic diseases, and Tuberous Sclerosis Complex (TSC) that I investigated the mechanism by which polycystin-1, involved in Polycystic Kidney Disease (PKD), regulated TSC2 (tuberin) a tumor suppressor mutated in TSC. This is when I found my way into the world of renal cell carcinoma and was intrigued as to the mechanisms by which early loss of chromosome 3p and co-deletion of several tumor suppressors contributed to renal cell carcinoma (RCC). We linked loss of primary cilia in VHL (von Hippel Lindau) disease to the activation of a -catenin and Aurora kinase A (AURKA) signaling pathway. Most recently, we made the discovery of AURKA as a direct and likely oxygen-independent target of VHL which was hugely exciting as it established a function for VHL in both normoxia and hypoxia. Given the role of VHL in regulating microtubules and our recent findings establishing the novel role of the epigenetic modifier SETD2 (Set-domain containing 2) in methylating microtubules, we are currently focusing on understanding and targeting novel pathways downstream of VHL that could rescue cytoskeletal defects in RCC. My interests in crosstalk between pathways and proteins involved in renal cell carcinogenesis, has led to several collaborations one of which I will be detailing in this talk.

Abstract: The tumor suppressor p27, a nuclear cyclin-dependent kinase (Cdk) inhibitor, is often mislocalized to the cytoplasm in cancers such as endometrial cancer (EC) and renal cell carcinoma (RCC). Cytoplasmic p27 serves an oncogenic role actively promoting tumorigenesis and is considered an adverse prognostic marker in solid tumors. We used a novel strategy targeting the interaction pocket of the E3 ubiquitin ligase (Skp2/Ck1) responsible for binding nuclear p27 and promoting p27 turnover. An innovative in silico predictive modeling approach was utilized to virtually screen small molecules for their ability to bind the Skp2-p27 interaction pocket, predicting an inability of this ubiquitin ligase to ultimately degrade p27.

The virtual screen identified 5000 ‘hits’ which were individually evaluated for their docked conformation, binding affinities, and chemotypes leading to the selection of 27 unique compounds. The virtual ‘hits’ combined with chemically similar molecules were subject to a high-content screening assay to identify probes that exclusively lead to elevated nuclear p27 without increasing cytoplasmic p27 levels. Five small molecules were ultimately chosen from this primary screen to advance through a battery of secondary biochemical validation assays. We confirmed the ability of these probes to increase nuclear p27, decrease p27 ubiquitination and inhibit cell cycle progression. These studies lead to the identification of Compound 276 which progressed to preclinical pharmacokinetic and pharmacodynamic studies at the CPRIT-funded core at Texas Southern University. These studies highlighted the feasibility of in silico modeling to screen a lager cohort of small molecules that specifically abrogated Skp2 binding to p27, which informed subsequent primary and secondary screens that identified a small molecule with potential value for clinical intervention.

Veronica Ajewole, PharmD, BCOP
Assistant Professor
Clinical Pharmacy Practice

Trends of Oral Formulation in Cancer Care: Focus on Capecitabine

Dr. Veronica B Ajewole earned her BS in Biochemistry from University of Ado Ekiti in Nigeria after which she worked as a Quality Control Personnel in an ISO-Certified Large-Scale Drug Manufacturing company for a few years before relocating to the United States in 2009. She completed her Doctor of Pharmacy degree with a Summa Cum Laude from Texas Southern University in 2015. After this, she completed her Post-Graduate Year 1 Pharmacy Residency and Year 2 Oncology pharmacy residency training at Houston Methodist Hospital in 2016 and 2017 after which she assumed the role of an Assistant Professor of Pharmacy Practice at Texas Southern University. Currently, she is a Board-Certified Oncology Pharmacist with Clinical Pharmacy practice privilege at Houston Methodist Hospital Cancer Center, Texas Medical Center location where she implemented a medication therapy management program for cancer patients on oral chemotherapy to improve overall clinical outcome and address cancer health disparities among this population.

Dr. Ajewole's didactic teaching expertise is in diverse oncology-related topics for professional students in the College of Pharmacy and Health Science as well as development/implementation of a pharmacy practice driven integrated lab experience for 2nd year professional PharmD students. She is a seasoned clinical preceptor and mentor for several students and residents. She is an author of publications in peer-reviewed journals and a reviewer for peer-reviewed articles. She has had several poster and platform presentations in local and national conferences. TSU recently received a 5-year $8.63 million grant award from the National Institute of Health's National Institute on Minority Health and Health Disparities to establish a Center for Biomedical and Minority Health Research (CBMHR) where Dr. Ajewole will serve as the director of the Community Engagement Core. Through $250,000 grant award from Centers for Medicare and Medicaid Services Minority Research Grant Program Dr. Ajewole and her team will implement a project titled "Development of predictive model and social determinants-based interventions for aggressive prostate cancer among Africa-American".

Texas Southern University

Dr. Ajewole and her team received Susan G Komen community grant to establish Breast Cancer Screening and Prevention Center at TSU. She is also leading a proposal to reformulate capecitabine to enhance the PK/PD parameters which will lead to improved patient compliance, medication adherence, and decreased side effects.

Dr. Ajewole is happily married to her loving husband and blessed with 3 wonderful children.

Abstract: **Background:** The increase of oral chemotherapy agents offers numerous advantages; however, it creates a unique set of challenges to providing optimal patient care in patients following a complex treatment plan. The pharmacist's interventions to conduct medication therapy management can improve adherence and clinical outcomes of oral chemotherapy agents such as capecitabine. Capecitabine was FDA approved in 1998 with indications for Metastatic Breast and Colon Cancer as well as adjuvant treatment of Colon Cancer. It is available as 150 mg and 500 mg oral tablets and usually dosed as 1250 mg/m2 twice daily orally for 2 weeks followed by a one-week rest period in a 3-week cycle. There is a significant drug-food interaction because food reduces both the rate and extent of its absorption. An average patient with a total body surface area of 2 m2 will take 2500 mg of capecitabine twice daily, equivalent to five 500-mg tablets 2 times a day. This is a high pill burden that can cause medication non-adherence for patients over time.

Objectives: Objective 1:To use Medication Therapy Management (MTM) in an outpatient cancer clinic setting to increase patient's adherence to capecitabine and optimize clinical outcome through patient education, adherence tracking, toxicity identification and management, decreasing polypharmacy, and potential drug interactions. Objective 2: To develop a poly lactic-co-glycolic acid (PLGA) formulation of capecitabine and determine the in-vivo and in-vitro drug release profile of the new formulation. Development of PLGA formulation of capecitabine as a controlled release drug delivery system will increase bioavailability and improve pharmacokinetic profile which will potentially decrease pill burden to enhance medication adherence.

Method(s) or Procedure(s): Objective 1 was a single-center chart review study at the Houston Methodist Hospital Outpatient Cancer Center. Objective 2 was done in collaboration with Dr. Huan Xie in CCPF facility at TSU.

Result(s): Toxicity assessments indicate patients post-MTM implementation are experiencing decreased toxicities after starting their capecitabine therapy due to pharmacist education and intervention provided to patients at baseline. When comparing groups, there is a 38% increase in pharmacist education provided to patients at the start of capecitabine therapy Pre-MTM implementation to post-MTM implementation. Among 41 patients on capecitabine in the post MTM implementation phase, clinical pharmacist made clinically significant interventions for 12 (29%) patients.

Reformulation studies was started but pending completion due to campus closure from COVID-19 pandemic. Preliminary data on PLGA reformulation study is promising and further in vivo PK study is ongoing.

Conclusion(s): Based on data analysis, we have concluded that our hypothesis was accepted and implementation of MTM services positively impacted adherence and patient health outcomes.

Disclosure(s): Veronica Ajewole, PharmD, BCOP is an Assistant Professor of pharmacy practice at Texas Southern University and received RCMI pilot grant funding to support this initiative. NIH/NIMMHD RCMI fund number: Project Number G12MD007605 (renewed award U54MD007605 and was also funded in part by Cancer Prevention & Research Institute of Texas (CPRIT) Core Facilities Support Awards under Award Number RP180748.

Yun Zhang, PhD
Assistant Professor
Pharmaceutical Sciences

Overview of the PD Facility of the CCPF

Dr. Yun Zhang is an Assistant Professor in the Department of Pharmaceutical Sciences at Texas Southern University (TSU). She obtained her Ph.D. in Biophysics from Columbia University and had her postdoctoral training in Cancer Genetics at the UT MD Anderson Cancer Center. As a mouse geneticist, Dr. Zhang has abundant experience of establishing mouse cancer models by genetic engineering or xenograft. Her current major research at TSU is to investigate the age-dependence and cell-specificity of breast cancer driven by mutant p53. She also studies HER2 positive breast cancer and is trying to understand the cooperation between different mutant p53 and HER2 in breast cancer development.

Abstract: The PD facility of the CCPF has the capability to measure the efficacy of candidate anti-cancer drugs using various in vitro and in vivo assays. The facility can, upon request by investigators, perform in vitro assays to measure how candidate drugs could affect cell proliferation, apoptosis, DNA damage or cell migration/ invasion. The PD facility also offers evaluation of drug efficacy on tumor development in vivo using xenograft assays in immunodeficient mice. In addition, we can also assist investigators with using genetically engineered mice that model a variety of cancers such as breast, pancreatic, colon and testes malignancies. If needed, we can also follow through to examine or identify biomarkers or proteins of interest in the tumors using immunoblotting, immunohistochemistry and/or immunofluorescence assays. These PD services provided by CCPF are invaluable for researchers who are interested in testing the efficacy of their candidate anti-cancer drugs but have limited expertise or resources to perform PD studies on their own. The comprehensive list of services offered by the PD facility is listed on the GCC CCPF website (https://www.gcc-ccpf.com).

The GCC CCPF is supported by CPRIT-Core Facilities Support Awards RP180748.

Stephan Schmidt, B.Pharm, PhD, FCP
Certara Professor
Associate Professor & Director
Center for Pharmacometrics & Systems Pharmacology
Department of Pharmaceutics Lake Nona

Model-Informed Drug Development: Challenges and Opportunities

Stephan Schmidt is an endowed Associate Professor in the Department of Pharmaceutics at the University of Florida, where he also serves as the Director for the Center for Pharmacometrics and Systems Pharmacology. He received his B.S. in Pharmacy from the Friedrich-Alexander University in Erlangen, Germany, and his PhD in Pharmacy from the University of Florida in Gainesville, USA. Following a post-doctoral fellowship at the Leiden-Amsterdam Center for Drug Research, he rejoined the University of Florida as faculty in 2012. Dr. Schmidt's research focuses on chronic progressive diseases, special patient populations, and drug-drug interactions. He published more than 100 peer-reviewed scientific manuscripts, 7 book chapters, and 2 textbooks, including the fifth edition of Rowland and Tozer's Clinical Pharmacokinetics and Pharmacodynamics textbook. He received numerous awards including the University of Florida Excellence Award for Assistant Professors in 2013, the Tanabe Young Investigator Award from the American College of Clinical Pharmacology (ACCP) in 2016, and the Outstanding Doctoral Thesis Mentoring Award from UF's College of Pharmacy in 2018. Dr. Schmidt serves as the Chair of the Special Interest Group on Precision Medicine of the International Pharmaceutical Federation (FIP), Subject Editor for the European Journal of Pharmaceutical Sciences, and Board of Regents member of ACCP.

Modeling & simulation (M&S) tools have long been used in engineering and aerospace industries to develop products that would be prohibitively expensive to optimize through iterative improvement of prototypes. Modern drug development is now adapting and integrating analogous tools based on information from all phases of the development process since it is neither cost-effective nor time-efficient to tackle all open questions experimentally. As a result, an increasing number of decisions are now based on M&S, a process which is now referred to as model-informed drug development (MIDD). However, prospective identification of clinically relevant sources of variability remain a challenge. To overcome this challenge the integrated use of multidisciplinary research approaches is needed. The objective of this keynote lecture is to provide an overview of M&S approaches and their application at different stages in drug development and regulatory evaluation through the use of selected case examples.

Carly Filqueira, PhD
Assistant Professor
Nanomedicine and Cardiovascular Surgery

Characterization and Optimization of Thyroid Hormone Metabolites for Type 1 Diabetes Treatment

Dr. Carly Filgueira obtained her Bachelor of Science degree in Chemistry Magna Cum Laude from The George Washington University in Washington, DC and obtained her masters and doctorate degrees in Chemistry from Rice University. Dr. Filgueira joined Houston Methodist in 2011 where she focused on nuclear hormone receptors and small molecule screening using a combination of direct binding and cell based assays. Dr. Filgueira is currently a member of the Department of Nanomedicine with appointments as Assistant Member in the Research Institute, Assistant Professor of Nanomedicine in the Academic Institute, and with the Department of Cardiovascular Surgery.

Abstract: Diabetic patients often also exhibit thyroid dysfunction and individuals with low and low-normal thyroid function are at a higher risk to develop diabetes. In the case of pediatric patients with T1D, hypothyroidism occurs much more than the general pediatric population (3 to 30 % versus 0.1 to 2 %). Clearly, these two endocrine disorders have a complex and intertwined relationship. Our lab is investigating the effects of treatment with thyroid hormone metabolites as a means to improve insulin production. In this talk we discuss the characterization and optimization of thyroid hormone metabolites as a novel approach to treat type 1 diabetes.

Dong Liang, PhD
Chair, Pharmaceutical and
Environmental Health Sciences
Professor, Pharmaceutics

Characterization and Optimization of Thyroid Hormone Metabolites for Type 1 Diabetes Treatment

Dr. Liang, Professor of Pharmaceutics, Texas Southern University, received his B.S. (pharmacy) and M.S. (pharmaceutics) from Zhejiang Medical University, and Ph.D. (pharmaceutics) from the University of Houston. His research interest is in pre-clinical and clinical phase I pharmacokinetics (PK) and pharmacodynamics (PD) studies in supporting drug development. He is co-inventor of five U.S. patents in dosage formulation development. He was formerly a research scientist at Mylan Pharmaceuticals Inc. He is the Director of CCPF in supporting preclinical cancer drug development.

Elisabeth Martinez, PhD
Assistant Professor
Pharmacology

The Multiple Activities of Jumonji Inhibitor JIB-04

Dr. Elisabeth Martinez received her Ph.D. in Biochemistry and Molecular Biology from Georgetown University where she studied the role of nuclear receptors in transcriptional regulation and went on to do a postdoctoral fellolwship at the National Cancer Institute at NIH. There she worked on steroid receptors, epigenetics and drug discovery. She joined the faculty at UT Southwestern's Department of Pharmacology where she currently is a tenured Associate Professor. Her scientific program at UT Southwestern is built upon a combined approach of defining the function of epigenetic enzymes and developing chemical tools to modulate their function. This dual strategy gives her lab the great advantage of simultaneously advancing basic knowledge and generating chemical probes with therapeutic potential. Their main interest is to molecularly define and pharmacologically target the pathological epigenetic and transcriptional events that characterize cancers and other human diseases, while uncovering new biology. For some years, their research has focused on Jumonji histone demethylase enzymes. Within the context of cancer, her group has discovered novel roles for Jumonji enzymes in transcriptional adaptation and reprogramming, in the development of chemotherapy resistance, in DNA repair pathways and in the response to radiation therapy in multiple tumor types. These findings have important implications for disease treatment, opening up new options for overcoming and preventing therapeutic resistance. The small molecule inhibitors they develop have in vivo efficacy without toxicity and have been excellent tools for gaining mechanistic insights into the Jumonji-driven molecular events driving cancer. In addition to chemical biology and drug discovery, her lab uses genetic, molecular and genomic approaches to understand the underlying biology and catalytic activity of these enzymes.

Abstract: Lysine histone demethylases (KDMs) of the Jumonji family are highly upregulated in multiple solid and hematological malignancies and drive oncogenic gene expression patterns. We have uncovered novel roles of Jumonji KDM enzymes in mediating transcriptional reprogramming during the development of resistance to chemotherapy and in enhancing DNA repair in response to radiation. We will present data using both genetic and pharmacological approaches to block the activities of Jumonji demethylases in cells and in vivo. Our findings strongly suggest that inhibition of enzymatic activity is an effective strategy to treat chemoresistant tumors and to prevent the development of drug resistance.

Furthermore, we have identified a role for Jumonji enzymes of the Jarid subfamily in DNA repair, by their modulation of H3K4 methylation at sites of DNA damage. In this context, we will report on the radiosensitizing effects of Jarid inhibition in radiation resistant cancer cells and tumors in vivo. Validation of our findings in human populations will also be presented. Our work opens a new paradigm in our mechanistic understanding of how histone demethylases mediate drug and radiation resistance and offers a therapeutic strategy to overcome these obstacles.

Song Gao, PhD
Assistant Professor
Pharmaceutical and Environmental Health Sciences

Alleviating Irinotecan-induced Diarrhea with Herbal Mixture Xiao-Chai-Hu-Tang (XCHT)

Song Gao, Ph.D. Assistant Professor, Department of Pharmaceutical and Environmental Health Sciences, TSU. Dr. Gao has broad expertise in the areas of pharmaceutics with specific training in drug absorption using the Caco-2 cell culture model/intestinal perfusion model, metabolism using microsome-mediated models, and drug bioanalysis using LC-MS/MS. He has been working on ADME of bioactive natural products for more than 10 years and has published more than 50 publications. Dr. Gao's research has been focusing on developing recycled locally bioavailable drugs for the treatment of chronic diseases in the colon and alleviating drug induced colonic side effects. His research has been funded by CPRIT and NIH.

Abstract: Irinotecan, a prodrug of SN-38, is used to treat many types of metastatic and drug-resistant cancers, and often represents the therapy of the last resort. Unfortunately, a large percentage (up to 40%) of these patients will experience serious (Grade 2) and severe (Grade 3-4) delayed-onset diarrhea (SDOD). SDOD, which cannot be effectively managed using current therapies. SDOD may lead to prolonged hospitalization and even death in some instances. The purpose of this study is to determine the mechanism of irinotecan-induced SDOD and determine the efficacy and mechanism of Xiao-Chai-Hu-Tang (XCHT), an herbal mixture, against irinotecan-induced SDOD. In vitro and in vivo mouse and rat models were used to determine the efficacy and mechanism. The results showed that SN-38 downregulated UGT1A1, a major enzyme catalyzing SN-38's glucuronidation, in the intestine to cause SN-38 accumulation in the colon, resulting in diarrhea. Additionally, the results showed that XCHT can selectively restore UGT downregulation caused by SN-38 without affecting liver UGT expression. Intestinal perfusion showed that XCHT can also inhibit biliary secretion of SN-38. Efficacy study showed that XCHT can effectively attenuated irinotecan-induced diarrhea without affecting therapeutic efficacy. We conclude that XCHT is a promising agent to alleviate irinotecan-induced diarrhea.

Poster abstracts in alphabetical order

First Name	Last Name	Institution	Abstract Title
Lorita	Agu	UH	*Population Pharmacokinetics of Vincristine and its Metabolite in Kenyan Pediatric Cancer Patients*
Dinh	Bui	UH	*PBPK Modelling of Matrine in Healthy Adults and Buccal Delivery Formulation for Prevention of Oral Cancer*
Nolan	Dvorak	UTMB	*Rationally Designing Peptidomimetics Derived from the β12 Sheet and β8-β9 Loop of Fibroblast Growth Factor 14 (FGF14) to Develop Allosteric Modulators of the Voltage-Gated Na^+ Channel 1.6*
Angel	Garces	MDACC	*The Effects of $ATRX^{Loss}$ and $IDH1^{R132H}$ when Combining Radiotherapy with the $IDH1^{R132H}$ Inhibitor Ivosidenib (AG-120) in Glioma Stem Cells*
JyotsnaDevi	Godavarthi	TSU	*Splicing Factor 1 (SF1) Levels Influence Colon Polyp Developent in Mice*
Pavani	Gonnabathula	TSU	*Removal of Trace Levels Emerging Contaminants (ECs) From Water By Green Synthesized Acalypha Indica Silver Nanoparticles*
Ritu	Gupta	TSU	*In Vitro Dissolution Considerations Associated with Nanoparticle-based Drug Delivery Systems (NDDS)*
Alex	Kang	RU	*Identification and Validation of a Novel Antivirulent that Binds to Pyoverdine and Inhibits its Function*
Victor	Lincha	UH	*Co-modeling of Calcipotriol and Paclitaxel in Blood and Tumor of KrasG12D Mouse Model of Pancreatic Cancer Post I.V. Dose of Micellar Co-formulation*
Maria	Nigro	TSU	*Development and Validation of a Sensitive, Specific and Reproducible UPLC-MS/MS Method for Quantification of a Potent Inhibitor of Methionine Aminopeptidase*
Svetlana	Panina	RU	*Mitophagy-Inducing Compounds as Potential Agents for Leukemia Eradication*
Jingqi	Pei	RU	*Utilizing Synergistic Potential of Mitochondria-Targeted Drugs for Leukemia Therapy*
Alexey	Revtovich	RU	*Novel Immune Modulators Enhance Caenorhabditis elegans Resistance to Multiple Pathogens*
Aditya	Singh	UTMB	*Development of Allosteric Modulators of the Voltage-Gated Sodium Channel 1.1*
Aditya	Singh	UTMB	*Peptide Mimicking Crucial Residues of the β12 Sheet of Fibroblast Growth Factor 14 Modulates Accumbal Excitability via Interactions with the Voltage-Gated Na^+ Channel 1.6*
Joe	Tolar	RU	*Characterizing Novel Mitochondria-Targeting Small Molecule Drugs in Caenorhabditis elegans*

First Name	Last Name	Institution	Abstract Title
Paul	Wadsworth	UTMB	*Developing Pharmacological Probes for Interrogation of the Circuitry of the Nucleus Accumbens*
Yang	Wang	TSU	*A UHPLC-MS/MS Method for The Quantification of JIB-04 in Rat Plasma: Development, Validation and Application to Pharmacokinetics Study*

Population Pharmacokinetics of Vincristine and its Metabolite in Kenyan Pediatric Cancer Patients

Agu L[1], Wu L[1], Renbarger J[2], Skiles J[2], Masters A[2], Chow D S-L[1]

1. Department of Pharmacological and Pharmaceutical Sciences, College of Pharmacy, University of Houston, Houston, Texas.
2. Department of Pediatrics, Indiana University School of Medicine, Indianapolis, Indiana

Corresponding Author: Lorita Agu, Department of Pharmaceutical and Pharmacological Sciences, College of Pharmacy, University of Houston, 4849 Calhoun Road, Houston, TX 77204; E-mail: loritaikodiya@gmail.com

Background: Vincristine (VCR) dosing strategies are largely empirical regardless of its extensive use in pediatric oncology. Little information is known about its disposition and optimal therapeutic dosing. Renbarger and associates reported that CYP3A5 enzyme metabolizes VCR to M1 more efficiently than CYP3A4 enzyme. This may be clinically significant as CYP3A5 expression varies among Kenyans (90%), African Americans (AA, 70%) and Caucasians (10-20%).

Hypothesis/Goals: It is essential to characterize the disposition of M1 in humans to provide an insight of the inter-ethnic variability in VCR metabolism and clearance, which will be helpful for future rational dosing regimen optimization. Pharmacokinetic (PK) dispositions of VCR and its M1 metabolite were characterized through PK co-modeling for Kenyan pediatric cancer patients with acute lymphoblastic leukemia or nephroblastoma.

Methods: *Pharmacokinetic Study:* Dried blood spot (DBS) samples were collected, via finger stick at various time points depending on the feasibility, from 25 Kenyan pediatric cancer patients (13 males/12 females, 1-14 years, BSA of 0.36 - 1.3 m^2) after an IV dose of VCR (1.6 – 3.1 mg/m^2). Concentrations of VCR and M1 from DBS were simultaneously quantified using a validated LC-MS-MS assay. *Pharmacokinetic Modeling:* A population PK (pop PK) co-model was developed using Phoenix® NLME™ to simultaneously capture and predict the PK of VCR and M1 concentrations. A correlation between the observed and predicted concentrations of VCR and M1 PK was established by pop PK fitting. Model discrimination was performed by visual inspection, goodness of fit plots and AIC value.

Results: The best fit pop PK co-model for VCR and its M1 metabolite was established. PK parameter estimates were derived. Volumes of distribution for VCR and M1 were 0.12 L (33.55 %CV) and 249.07 L (33.42 %CV), respectively. The elimination rate constants for VCR and M1 were 5.6 hr^{-1} (22.81 %CV) and 0.00015 hr^{-1} (66.85 %CV), respectively. The conversion rate constant of VCR to its M1 metabolite was 9.66 hr^{-1} (32.49 %CV). Clearance values were 0.67 L/hr for VCR and 0.04 L/hr for M1.

Conclusions: VCR and its M1 metabolite exhibit distinct PK characteristics, in that M1 distribution is substantially larger than that of VCR (249 vs 0.12 L), and clearance is slower than that of VCR (0.04 vs 0.67 L/hr). The conversion kinetics of VCR to M1 is characterized for the first time, which may potentially offer a rationale for ethnic disparity in VCR therapy. In addition, a large interpatient variability is documented even within the same ethnic Kenyan population. After model refinement and validation, our model could potentially serve as a tool for rational VCR dosing regimen modification for Kenyan/AA pediatric cancer patients.

Acknowledgements: Funding from Institute for Drug Education and Research (IDER), University of Houston, College of Pharmacy; 2020 AACR Scholar-in-Training Award

PBPK Modelling of Matrine in Healthy Adults and Buccal Delivery Formulation for Prevention of Oral Cancer

Bui D[1], Singh R [1], You M[2], Hu M[1]

1. Department of Pharmacological and Pharmaceutical sciences, College of Pharmacy, University of Houston
2. Department of Pharmacology and Toxicology, Medical College of Wisconsin

Corresponding author: Dinh Bui, Department of Pharmacological and Pharmaceutical Sciences, College of Pharmacy, University of Houston, 4849 Calhoun Rd, Houston, TX 77204, E-mail: dtbui5@central.uh.edu

Background

Oral cavity carcinomas are the sixth most common cancer in the world, approximately 600,000 new cases are diagnosed every year with a mortality rate of 40-50%. Antitumor B (ATB, Zeng Sheng Ping, ACAPHA), is a natural herbal supplement composed of six plants. Recent clinical studies showed that ATB is active against oral cancer. Matrine (Matr) was listed as the quality control marker of Antitumor B (ATB). However, little information has been known about the pharmacokinetics of ATB in human.

Hypothesis/Goals

The aims of this study are (1) to develope a PBPK model and characterize the pharmacokinetics profile of Matr in healthy adults and (2) an alternative formulation is needed to enhance the delivery of ATB to the targeted oral tissues efficiently.

Method

An open-label PK study of ATB 2400 mg single dose was conducted on 8 healthy adults to investigate the safety and PK of ATB compounds in healthy adults. The study was approved by the IRB of University of Houston, Texas, USA. Each participant took 8 ATB tablets of 300 mg each with 250 mL water after overnight fasting. Plasma and saliva samples were collected pre-dosing, 0.5, 1, 2, 3, 4, 6, 8 and 24 hrs post dosing. The GastroPlus® 9.7 and PK-Sim 8.0 were used to build the PBPK model for Matr. The buccal delivery patch of ATB was prepared using solvent casting method.

Results

ATB was found to be safe after oral administration of a single dose 2400 mg on 8 healthy volunteers. Matrine was detected at quantifiable concentrations in human plasma and saliva. Significant higher concentration of Matr in the saliva sample suggested an active secrection of this compound to saliva. The PBPK model adequately described Cp-time profiles of Matr. The developed PBPK model adequately captured the observed Matr Cp time profile following oral administration under fasted state in healthy subjects. The formulation could release drug within 30 mins.

Conclusion

This study contributes to the pharmacokinetics of Antitumor B herbal supplement in healthy adults. The plasma concentration of matrine were adequately described using physiological and drug-specific parameters. The model successfully captured the dose and time-dependent PK of Matr in human plasma. Pharmacokinetics of Matr could be tracked with saliva sampling. More information of the transporter in the salivary gland compartment is needed for the model to be extended for quantitative prediction of the drug concentration in saliva. The buccal patch formulation is promising to locally deliver ATB to the target site.

Rationally Designing Peptidomimetics Derived from the β12 Sheet and β8-β9 Loop of Fibroblast Growth Factor 14 (FGF14) to Develop Allosteric Modulators of the Voltage-Gated Na^+ Channel 1.6

Dvorak NM, Wadsworth PA, Wang P, Singh AK, Chen H, Zhou J, Laezza F
Department of Pharmacology and Toxicology, University of Texas Medical Branch

Corresponding author: Fernanda Laezza, Department of Pharmacology and Toxicology, University of Texas Medical Branch, 301 University Blvd, Galveston, TX, Email: felaezza@utmb.edu

Background: Disruption of protein:protein interactions (PPI) between voltage-gated Na^+ (Na_v) channels and their auxiliary proteins causes channelopathies. Despite restoration of these perturbed PPIs serving as a novel therapeutic approach, efforts to develop such PPI modulators are vexingly difficult for two main reasons. Firstly, PPI interfaces are structurally divergent from the surfaces that most conventional small molecules are designed to target on account of their expansive size and relatively featureless topography. Secondly, insights into where along a PPI interface to target with a small molecule are typically hindered by a lack of structural information regarding motifs of binding partners that confer functionally relevant modulation.
Hypothesis/Goals: Focusing on the PPI between $Na_v1.6$ and its auxiliary protein fibroblast growth factor 14 (FGF14), we previously identified that the PLEV and EYYV motifs of the β12 sheet and β8-β9 loop of FGF14, respectively, are predicted to disproportionately contribute to the Gibbs energy of FGF14:$Na_v1.6$ complex assembly. Based upon these structural insights, peptidomimetics derived from these motifs were designed, synthesized, and pharmacologically evaluated in an effort to develop allosteric modulators of $Na_v1.6$.
Methods: Combinatorial chemistry, split-luciferase complementation assay (LCA), surface plasmon resonance (SPR), and whole-cell patch-clamp electrophysiology.
Results: Peptidomimetics derived from the PLEV motif were first pursued. In total, 30 analogs of the tetrapeptide were designed and synthesized. Of these analogs, nine were shown using the LCA to inhibit FGF14:$Na_v1.6$ complex assembly at low micromolar concentrations. Of these nine analogs, PW0564 stood out for its ability to bind to both FGF14 and the C-terminal domain (CTD) of $Na_v1.6$ using SPR. Subsequent functional evaluation of PW0564 as a modulator of $Na_v1.6$ channels using whole-cell patch-clamp electrophysiology in heterologous cells revealed that, in the absence of FGF14, PW0564 exerted phenotypes similar to those observed by co-expression of FGF14, indicating the ability of PW0564 to serve as a pharmacological mimic of the β12 sheet of FGF14. Having developed an allosteric modulator of $Na_v1.6$ that exerted its effects via pharmacological mimicry of the β12 sheet of FGF14, analogs of the EYYV motif were subsequently pursued. In total, 12 analogs of the motif were designed and synthesized. Of the 12 analogs, PW201 stood out for its ability to inhibit FGF14:$Na_v1.6$ complex at low micromolar concentrations and for its ability to bind to both the CTD of $Na_v1.6$ and FGF14. Similar to PW0564, subsequent functional evaluation of PW201 as a modulator of $Na_v1.6$ channels using whole-cell patch-clamp electrophysiology revealed that it served as a negative allosteric modulator (NAM) of $Na_v1.6$ by suppressing $Na_v1.6$-mediated peak current density.
Conclusions: Using combinatorial chemistry, in tandem with techniques for *in vitro* pharmacological evaluation, including LCA, SPR, and whole-cell patch-clamp electrophysiology, we have identified NAMs of $Na_v1.6$ derived from β12 sheet and β8-β9 loop of FGF14. With further chemical optimization to improve the drug-like properties of these analogs, it is envisioned that these pharmacological tools used for interrogating the biophysical properties of $Na_v1.6$ could be developed into PPI-based neurotherapeutics.
Acknowledgments: We acknowledge our funding sources as follows: NIH Grant R01MH095995 (FL), R01MH12435101 (FL) NIH Grant R01MH111107 (FL, JZ), John Sealy Memorial Endowment Funds (FL), R01 DA038446 (JZ), P30 DA028821 (JZ), CPRIT RP150578 (CS), PhRMA Foundation (PAW), and HAMBP training grant no. T32GM008280 (NMD).

The Effects of ATRX^{Loss} and IDH1^{R132H} when Combining Radiotherapy with the IDH1^{R132H} Inhibitor Ivosidenib (AG-120) in Glioma Stem Cells

Garcés AA[1,2], Bronk L[1], Bhat KP[3], Grosshans DR[1].
1. Department of Radiation Oncology, MD Anderson Cancer Center, Houston, TX.
2. Therapeutics and Pharmacology, MD Anderson UTHealth Graduate School of Biomedical Sciences, Houston, TX.
3. Department of Translational Molecular Pathology, MD Anderson Cancer Center, Houston, TX.

Corresponding Author: Ángel Adrian Garcés, Department of Radiation Oncology, MD Anderson Cancer Center, 6565 MD Anderson Blvd, Houston, TX 77030, E-mail: aagarces@mdanderson.org.

Background: Glioblastoma Multiforme (GBM) is the most prevalent and aggressive form of malignant glioma in the USA with only a 15 month median survival time.[1,2] The recent discovery of glioma stem cells (GSCs), a subpopulation of undifferentiated, self-renewable, tumor initiating cells in GBM tumors known to promote chemoradioresistance, metastasis, and tumorigenesis, presents a new target for therapeutic intervention.[3,4] Importantly, 70-80% of low-grade glioma (LGG) and secondary GBM patients harbor IDH1^{R132H}, a mutation that occurs early in tumorigenesis and results in the elevated production of 2-Hydroxyglutarate (2-HG), a metabolite that contributes to intracellular oxidative stress and impaired post-radiation DNA damage repair (DDR) via Homologous Recombination (HR).[5-9] Although IDH1^{R132H} is associated with improved patient survival and clinical response to chemotherapy,[10] astrocytoma (LGG) patients specifically also harbor ATRX^{Loss}, a genetic alteration that inactivates the ATRX chromatin remodeling protein and ultimately impairs DDR via non-homologous end joining (NHEJ).[6,11-13] Interestingly, the recent development of Ivosidenib, an IDH1^{R132H} inhibitor shown to impair tumor cell growth and promote GSC differentiation by inhibiting 2-HG production, represents a potential treatment modality to incorporate alongside conventional X-ray radiotherapy in glioma patients.[14,15] However, the mechanisms by IDH1^{R132H} and ATRX^{Loss} impact the efficacy of X-ray radiotherapy (XRT) when combined with Ivosidenib in GSCs are not well understood. **We hypothesize that ATRX^{Loss} is required to successfully sensitize GSCs to the cytotoxic effects of XRT in the presence of Ivosidenib.**

Methods: Isogenic MGG18-IDH1^{WT}, MGG18-IDH1^{R132H}, TS543-ATRX^{WT}, TS543-ATRX^{Loss}, and GS818-IDH1^{R132H}/ATRX^{Loss} GSC neurospheres were treated with 3-6 Gy XRT using the XRAD 320 irradiation platform and/or 5 nM Ivosidenib, at 24 hours prior to irradiation. GSC self-renewal, based on neurosphere formation frequency, was quantified using extreme limiting dilution analysis (ELDA).[16]

Results: ATRX^{Loss} or IDH1^{R132H} alone were not sufficient to inhibit GSC self-renewal after XRT irradiation compared to ATRX^{WT} or IDH1^{WT} respectively (Pairwise Testing $Pr(>\chi^2)<0.0001$). The combination of XRT and Ivosidenib in MGG18-IDH1^{R132H} GSCs promoted XRT resistance by increasing self-renewal compared to XRT or Ivosidenib alone (Pairwise Testing $Pr(>\chi^2)<0.01$). Finally, we demonstrated that combining XRT and Ivosidenib only impairs self-renewal compared with Ivosidenib alone (Pairwise Testing $Pr(>\chi^2)<0.0001$) in GS818-ATRX^{Loss}/IDH1^{R132H} GSCs.

Conclusions: We conclude that ATRX^{Loss} is required for the GSC sensitivity to XRT and Ivosidenib, thereby supporting the benefits of this regiment for astrocytoma patients. Our future experiments will further elucidate the mechanisms by which XRT and Ivosidenib impact GSC apoptosis and DNA damage.

Acknowledgements: A. Garcés acknowledges Drs. Grosshans, Bhat, Guan, Sawakuchi, Lin, and Bronk for their continued mentorship and support throughout this project. GSCs were generously donated to A. Garcés by Dr. Krishna Bhat and Dr. Jason Huse at MD Anderson Cancer Center as well as Dr. Daniel Cahill at Massachusetts General Hospital. This research was funded by the NIH R01 grant entitled "Synaptic basis of deficits in attention and executive function following cranial radiation."

References

1. Mesfin FB, Al-Dhahir MA: Cancer, Brain Gliomas, StatPearls. Treasure Island (FL), StatPearls Publishing

2. Tamimi AF, Juweid M: Epidemiology and Outcome of Glioblastoma, in De Vleeschouwer S (ed): Glioblastoma. Brisbane (AU), Codon Publications

3. Liebelt BD, Shingu T, Zhou X, et al: Glioma Stem Cells: Signaling, Microenvironment, and Therapy. Stem Cells Int 2016:7849890, 2016

4. Arnold CR: The role of cancer stem cells in radiation resistance. 2020

5. Philip B, Yu DX, Silvis MR, et al: Mutant IDH1 Promotes Glioma Formation In Vivo. Cell Rep 23:1553-1564, 2018

6. Sulkowski PL, Corso CD, Robinson ND, et al: 2-Hydroxyglutarate produced by neomorphic IDH mutations suppresses homologous recombination and induces PARP inhibitor sensitivity. Sci Transl Med 9, 2017

7. M Gagné L, Boulay K, Topisirovic I, et al: Oncogenic Activities of IDH1/2 Mutations: From Epigenetics to Cellular Signaling. Trends in cell biology 27:738-752, 2017

8. Alan Mitteer R, Wang Y, Shah J, et al: Proton beam radiation induces DNA damage and cell apoptosis in glioma stem cells through reactive oxygen species. Scientific reports 5:13961-13961, 2015

9. Malta TM, de Souza CF, Sabedot TS, et al: Glioma CpG island methylator phenotype (G-CIMP): biological and clinical implications. Neuro-oncology 20:608-620, 2018

10. Khurana R, Rath S, Singh HB, et al: Correlation of Molecular Markers in High Grade Gliomas with Response to Chemo-Radiation. Asian Pacific Journal of Cancer Prevention 21:755-760, 2020

11. Haase S, Garcia-Fabiani MB, Carney S, et al: Mutant ATRX: uncovering a new therapeutic target for glioma. Expert opinion on therapeutic targets 22:599-613, 2018

12. Nunez FJ, Mendez FM, Kadiyala P, et al: IDH1-R132H acts as a tumor suppressor in glioma via epigenetic up-regulation of the DNA damage response. Sci Transl Med 11, 2019

13. Koschmann C, Lowenstein PR, Castro MG: ATRX mutations and glioblastoma: Impaired DNA damage repair, alternative lengthening of telomeres, and genetic instability. 3:e1167158, 2016

14. Rohle D, Popovici-Muller J, Palaskas N, et al: An Inhibitor of Mutant IDH1 Delays Growth and Promotes Differentiation of Glioma Cells. Science 340:626-630, 2013

15. Popovici-Muller J, Lemieux RM, Artin E, et al: Discovery of AG-120 (Ivosidenib): A First-in-Class Mutant IDH1 Inhibitor for the Treatment of IDH1 Mutant Cancers. ACS Medicinal Chemistry Letters 9:300-305, 2018

16. Hu Y, Smyth GK: ELDA: Extreme limiting dilution analysis for comparing depleted and enriched populations in stem cell and other assays. Journal of Immunological Methods 347:70-78, 2009

Splicing Factor 1 (SF1) Levels Influence Colon Polyp Development in Mice

Godavarthi JD*, Polk S*; Lisa Nunez*; Shivachar A*, Glenn Griesinger NL[#] , Matin A[*#]

Affiliation: *Dept. of Pharmaceutical Sciences, Texas Southern University, Houston, TX; [#]Dept. of Mathematics, Texas Southern University, Houston, TX.

[*#]**Corresponding author:** Angabin Matin, 3100 Cleburne Street, Houston, Texas,

Angabin.Matin@tsu.edu

Background:
Colorectal cancer (CRC) is the third most common cancer in the US, with the second highest in terms of mortality rates, and with higher incidence and mortality rates in African Americans. Although great progress has been made in the screening and detection of this cancer, there are still many aspects to be elucidated regarding the genetic susceptibility factors of this cancer.
Splicing Factor 1 (SF1) is an alternative splicing (AS) factor that is ubiquitously expressed and conserved across species. It is involved in early spliceosome assembly and may function as a constitutive splicing factor in lower eukaryotes but as an alternative splicing (AS) factor in mammalian cells. It interacts with U2 snRNP auxiliary factor ($U2AF^{65}$) to co-operatively bind to the branch point sequence and polypyrimidine tract within the intron of pre-mRNAs during splicing.

Hypothesis/Goals:
Mutational inactivation of APC (adenomatous polyposis coli) is frequent in colon cancers. Our goal is to utilize mouse models to assess genetic factors that influence colon tumorigenesis. We developed a mouse strain *Sf1+/-* that expresses reduced levels of splicing factor 1 (SF1) protein. We tested whether lower levels of SF1 could affect APC mediated colon cancer.

Methods:
$Apc^{Min/+}$mice develop numerous intestinal polyps by 4 months. We collected 2 cohorts of mice: $Apc^{Min/+}$and $Sf1+/-;Apc^{Min/+}$ mice in which the number and sizes of intestinal polyps were counted.

Results:
Results showed that $Sf1+/-;Apc^{Min/+}$ mice had lower numbers of intestinal polyps compared to $Apc^{Min/+}$. Fewer, smaller sized polyps were detected in intestines of $Sf1+/-;Apc^{Min/+}$ but the numbers of larger sized polyps were similar in both cohorts. Thus, lowered SF1 levels reduced incidence of colon polyps, likely by decreasing the number of polyps that are initiated in the intestine.

Conclusions:
Our results show that congenitally reduced SF1 levels resulted in reduction in intestinal polyp development in $Apc^{Min/+}$ mice and also suggest that SF1 levels influence cellular transformation and polyp development.

Acknowledgements:
GRANT SUPPORT
NCI R21CA186033 and NIMHD 5G12MD007605

Removal of Trace Levels Emerging Contaminants (ECs) From Water By Green Synthesized Acalypha Indica Silver Nanoparticles

Gonnabathula PK, Yakubu MA

Email: p.gonnabathula0252@student.tsu.edu
Department of Environmental and Interdisciplinary Sciences, College of Science and Technology, Texas Southern University, Houston, TX 77004

Emerging contaminants (ECs) include but are not limited to water disinfectant byproducts, gasoline additives, manufactured nanoparticles, pesticides, herbicides, UV-filters, human and veterinary pharmaceutical products. They have been found in trace amounts (ppt to ppb) in treated wastewater and have been detected in water samples – they are therefore difficult to remove. We have synthesized silver nanoparticles (Ag NPs) using Acalypha indica leaf extracts; and use them to treat pesticides and hormones in water samples to determine efficiency of removal. Ag-herbal extract nanoparticles was synthesized by incubation of Acalypha Indica extract with $AgNO_3$ at 80^0 C for 1 hour until the change of color from pale yellow to dark brown indicated the formation of colloidal silver nanoparticles. The synthesized Ag NPs were characterized by UV-Vis spectrophotometer, Zetasizer (ZS 90, Malvern, UK) for particle size and zeta potential of the Ag NPs. To investigate the effects of the Ag NP's on the removal of ECs from water, a 6 ml portion of the silver nanoparticle solution was centrifuged at 10,000 rpm for 7 minutes. The supernatant was discarded and to the residue, 5 ml of 5 ppm and 10 ppm pesticide/hormone standard solution was added and then allowed for continuous shaking for 1 hour. Then centrifuge at 1500 rpm for 5 min. 1ml of supernatant solution was collected, samples were filtered using 0.45µm syringe filter into HPLC vials for analysis. This experiment was repeated and allowed for continuous shaking for 24 hours to measure ECs removal/degradation efficiency of Ag NP's using HPLC analytical methods. Our results indicated that the Ag NP synthesized showed Absorption spectrum (absorbance peak) at about 400 nm, which confirmed the formation of Ag NP. The average size and zeta potential analyzed by dynamic light scattering techniques (DLS) showed the sized to be 132.6 nm and the Zeta potential as -ve 13.6 mV suggesting higher stability of Ag NPs. After treatment there is a significant reduction in the peak areas observed in samples treated with A. indica Ag NP's. Among the selected hormonal compounds and pesticides, there is a 100% removal of 5ppm cortisone, aldrin, endrin and 10 ppm cortisone after 24 hours of treatment. After 1 hour of treatment 92% of 5 ppm cortisone, 75.5% of 10 ppm cortisone, 58.8% of 5 ppm endrin, 78% of 10 ppm endrin were removed. After 24 hours 47.7% of 5 ppm testosterone, 35% of 5 ppm atrazine, 58.2% of 10 ppm estrone, 37.7% of testosterone, 38.4% of 10 ppm atrazine, 66% of 10 ppm aldrin and 78% 10 ppm endrin were removed. This indicates the removal of ECs by Ag NP's depends upon the nature and concentration of ECs and the removal mechanism is time dependent. Overall, Ag NP's achieved 100% removal of some targeted ECs by simple treatment method. This is a novel effort that need further investigation for broader application in water treatment processes to remove contaminants and in environmental bioremediation.

Key words: Emerging Contaminants, Acalypha Indica, Silver Nanoparticles

Acknowledgement/funding: This research was partly supported by supplies funded by Tittle III Award Number P031B090216; Title III, Part B, Historically Black Graduate Institutions (HBGI) (CFDA No. 84.031B), United States Department of Education

In Vitro Dissolution Considerations Associated with Nanoparticle-based Drug Delivery Systems (NDDS)

Gupta R[1], Chen Y[1], Xie H[1*]
Department of Pharmaceutical Science, College of Pharmacy and Health Sciences, Texas Southern University, Houston, TX, USA 77004.

*Corresponding author: Huan Xie, Texas Southern University, Gray Hall 219, 3100 Cleburne Street, Houston, TX 77004, USA, Email: Huan.Xie@tsu.edu

Nanoparticle-based Drug Delivery Systems(NDDS) have emerged as a promising formulation approach for drug delivery, by increasing drug solubility and dissolution rates with large surface area-to-volume ratios, by improving passive diffusion across biological membranes with reduced particle size, and by utilizing liposomal- and lipid-based nanocarriers to enhance drug permeability across phospholipid cellular membrane. A widely accepted modeling tool in pharmacokinetic studies, *in vitro in vivo* correlation (IVIVC), is often used to predict *in vivo* pharmacokinetic profiles of a study drug by building mathematical models based on *in vitro* drug release in a dissolution apparatus.

However, significant challenges exist in IVIVC of NDDS due to incomplete understanding of nanoparticles' biochemical characteristics and how they impact experimental protocols. Unlike that of conventional dosage forms, the *in vivo* disposition of nanocarriers is highly intricate, differing greatly from their *in vitro* behavior due to the interplay between the materials and the local biological environment. An example would be the outset of spontaneous surface-adsorbed layer of biomolecules around the nanoparticles, termed as protein-corona (PC), that causes large deviation from traditional, well-studied pharmacokinetic profiles. Owing to numerous physicochemical characteristics of these nanoparticles (e.g. size, shape, surface charge, topology, hydrophobicity), standardization of *in vivo* nanoparticle interactions becomes difficult, especially when concerning phagocytic pathways. Therefore, to obtain reliable and realistic IVIVC modeling data, existing protocols must be executed under physiologically relevant *in vitro* conditions, which are not well described in available literature to date.

Characterizing drug safety profiles for nanoparticles is another aspect of this review. While NDDS release can be sustained for months to decrease the dosing frequency and reduce adverse events associated with concentration peaks, the long-term fate of study compounds, including elimination, accumulation, and biodegradation, should be scrutinized to develop safer nanomedicines.

Nanotechnology offers promising solutions to the development of potential drug candidates and to the maintenance of exclusivity after expiry of patents associated with referenced products. Hence, it becomes indispensable that we clearly understand the impact of bio-nano interactions, and design *in vitro* dissolution strategies capable of making accurate prediction of *in vivo* pharmacokinetic profiles. Reliable IVIVC will properly correlate nanoparticle physicochemical properties with its performance in a physiologic environment, with favorable economic implications. This review aims to describe various challenges associated with characterizing nanoparticle properties during *in vitro* dissolution studies, to elucidate the impacts of bio-nano interactions, and to propose strategies to develop reliable pharmacokinetic modeling of nanoparticles.

Acknowledgements-This study was funded in part by Cancer Prevention & Reseach Institute of Texas (CPRIT) Core Facilities Support Awards under Award Number RP180748 and the National Institute on Minority Health and Health Disparities of the National Institute of Health (NIH) under Award Number 2U54MD007605. The content is solely the responsibility of the authors and does not necessarily represent the official views of the CPRIT and NIH.

Identification and Validation of a Novel Antivirulent that Binds to Pyoverdine and Inhibits its Function

Kang D[1], Wang X[1], Revtovich AV[1], Kleerekoper Q[2], Kirienko NV[1]
1. Department of BioSciences, Rice University
2. Shared Equipment Authority, Rice University

Corresponding author: Natalia V. Kirienko, Department of BioSciences, Rice University, 6100 Main St., Houston, TX, E-mail: kirienko@rice.edu

Background: *Pseudomonas aeruginosa* is a multidrug resistant, nosocomial pathogen that frequently causes ventilator-associated pneumonia in intensive care units and chronic lung infections in cystic fibrosis patients. The rising prevalence of drug-resistant bacteria demands new therapeutic avenues to treat *P. aeruginosa* infections. The major siderophore pyoverdine is a key virulence factor in the pathogen that can be targeted for therapeutic intervention. Pyoverdine not only provides the bacterium with ferric iron, a micronutrient necessary for growth, but also regulates the production of secreted toxins. Furthermore, we have recently demonstrated that pyoverdine disrupts *Caenorhabditis elegans* iron and mitochondrial homeostasis even in the absence of live pathogen. Due to a combination of these factors, pyoverdine production is necessary for full *P. aeruginosa* virulence against various mammalian and invertebrate hosts.

Hypothesis/Goals: We hypothesized that inhibitors of pyoverdine function would attenuate the production of pyoverdine-regulated virulence factors and mitigate *P. aeruginosa* pathogenesis against *C. elegans*.

Methods: Pyoverdine functional inhibitors were identified using high-throughput biochemical screen that was based on quenching in intrinsic fluorescence of pyoverdine. Specifically, we measured fluorescence after partially purified pyoverdine was treated with approximately 45,000 compounds from small molecule diversity libraries. *C. elegans* host death upon *P. aeruginosa* exposure was visualized via fluorescence microscopy after staining dead organisms with cell impermeant dye. ^{1}H-^{15}N and ^{1}H-^{13}C 2D NMR were used to identify the inhibitor binding sites on pyoverdine. Molecular docking and molecular dynamic simulations were used to further analyze small molecule – pyoverdine interactions.

Results: Twelve hits were identified from the screen, and top three were chosen for initial follow-up using structure-activity relationship analysis. Having tested various chemical analogues of these hits, we identified PQ3c, an analogue that exhibited greater affinity towards pyoverdine than its parental compound. PQ3c was able to attenuate the production of pyoverdine-regulated virulence factors (the translational inhibitor exotoxin A and protease PrpL) and rescue *C. elegans* hosts during *P. aeruginosa* exposure. PQ3c did not exhibit overt toxicity against *C. elegans* or human bronchial epithelial cells. Using NMR spectroscopy, we were able to demonstrate that PQ3c occupies a shallow groove on pyoverdine formed by the chromophore and N-terminal residues of the peptide chain. Using molecular docking and molecular dynamics simulations, we modeled the putative pyoverdine-inhibitor complex and predicted its interactions with the pyoverdine outermembrane receptor FpvA.

Conclusions: Antivirulence therapeutics, such as pyoverdine functional inhibitors, can be identified from high-throughput biochemical screens. PQ3c may serve as a scaffold for the development of pyoverdine inhibitors with higher potency and specificity.

Acknowledgements: This study was supported by National Institute of Health grants K22AI110552 and R35GM129294, by the Welch Foundation Grant C-1930, and John S. Dunn Foundation Collaborative Research Award Program administered by the Gulf Coast Consortia.

Co-modeling of Calcipotriol and Paclitaxel in Blood and Tumor of Kras^{G12D} Mouse Model of Pancreatic Cancer Post I.V. Dose of Micellar Co-formulation

Lincha VR[1], Zhao J[2], Xiaoxia Wen X[2], Duong L[1] , Li C[2], Chow D S-L[1]
1. Department of Pharmaceutical and Pharmacological Sciences, College of Pharmacy, University of Houston, Texas.
2. Cancer Systems Imaging, The University of Texas M.D Anderson Cancer Center, Houston, Texas

Corresponding Author: Victor R. Lincha, Department of Pharmaceutical and Pharmacological Sciences, College of Pharmacy, University of Houston, 4849 Calhoun Road, Houston, TX 77204; E-mail: victorrlincha@gmail.com

Background: Stroma-modulating agents in combination with standard chemotherapy have emerged as a potential treatment for pancreatic cancer, and we are developing a combination therapy of Calcipotriol (Cal) and paclitaxel (PTX). Cal, a stroma-modulating agent de-activates the cancer associated fibroblasts, shifting the tumor microenvironment into a dynamic milieu that aids PTX delivery. However, Cal can cause hypercalcemia at high doses.

Hypothesis/Goals: To mitigate toxicity and further improve tumor accumulation, we developed a micellar co-formulation (M-Cal/PTX).

Methods: Orthotopic Kras mouse model was developed. Healthy and tumor bearing mice were administered an IV bolus dose of M-Cal/PTX through the tail vein (5 mg/kg PTX and 0.5 mg/kg Cal). Whole blood samples were taken, as well as tumor, liver and spleen samples were harvested from tumor bearing group at predetermined time points post dose for simultaneous PTX and Cal quantifications by using a developed and validated LC-MS/MS assay. Compartmental analysis was performed to compare PK in healthy and diseased animals. To better understand the distribution kinetics of M-Cal/PTX, we co-modeled blood and tissue concentrations using Phoenix NLME (version 8.0).

Results: Cal from M-Cal/PTX (M-Cal) was measurable in the tumors and livers but not blood in orthotopic murine Kras^{G12D} mouse model 24 h post the IV bolus dose (0.5 mg/kg Cal, 5 mg/kg PTX). In contrast, Cal was not measurable from non-formulated free Cal. PTX was measurable in blood at 24 h in both formulated and non-formulated groups, but only measurable in the tumors of mice administered M-Cal/PTX. Our analysis showed PK differences of M-Cal in normal versus tumor-bearing mice. The elimination half-life ($t_{1/2}$) of M-Cal in diseased mice was >4X shorter than that in healthy ones (0.089 h vs 0.38 h), which corresponded with the increased uptake into the peripheral tissues. M-Cal persisted in tumor and liver because of the slow eliminations from these organs. Unsurprisingly, our model estimated that the blood AUC in tumor-bearing mice was 5X less than that in the healthy counterparts. A similar analysis of PTX from M-Cal/PTX showed that the elimination $t_{1/2}$ was about 2X shorter in diseased mice when compared to their healthy counterparts (0.58 h vs 1.2 h). This corresponded with a modest blood AUC decrease in diseased mice. Of the three organs evaluated, our model estimated PTX liver uptake to be the highest, followed by the spleen and then tumor. The rates of eliminations from the three tissues varied with the tumor being the highest. PTX could be measured in all three sites 24 h after M-Cal/PTX injection.

Conclusions: We conclude that 1) PK differences exist for Cal and PTX from M-Cal/PTX between tumor-bearing and healthy mice with faster elimination in the former. 2) Reduced Cal AUC in blood and its presence in tumors are expected to potentially reduce systemic toxicity and increase efficacy profiles, respectively, because Cal toxicity is directly related to the elevated systemic exposure that exacerbates off-target vitamin D receptor activation. Future studies will focus on demonstrating the proof-of-concept therapeutic merits of our combination regimen in the orthotopic Kras^{G12D} mouse model of pancreatic cancer.

Acknowledgement: Gilson Longenbauch Foundation Funding; 2020 AACR Scholar-in Training Award

Development and Validation of a Sensitive, Specific and Reproducible UPLC-MS/MS Method for Quantification of a Potent Inhibitor of Methionine Aminopeptidase.

Rincon-Nigro ME*, Ma J, Awosemo OT, Olaleye OA, Liang D

Department of Pharmaceutical and Environmental Health Sciences, Texas Southern University

*Corresponding author: Maria Rincon-Nigro, Department of Pharmaceutical and Environmental Health Sciences, College of Pharmacy and Health Sciences, Texas Southern University, 3100 Cleburne Street, Houston, TX 77004, USA. m.rinconnigro7799@student.tsu.edu

Background: OJT007 is a potent and selective inhibitor of Methionine aminopeptidase 1 (MetAP1) with potent activity against *Leishmania Major (L. major,* the causative infectious agent of Cutaneous Leishmaniasis (CL). Inhibition of MetAP1 interferes with *L.major* amastigotes and promastigotes proliferation. This makes OJT007 a promising lead molecule for potential development of novel agents for the treatment of CL.

Goals: To facilitate the quantification of lead molecules during the different stages of drug development, it is necessary to develop a bioanalytical assay. The objective of this study is to develop and validate an LC-MS/MS method for the quantification of a novel leishmanicidal agent in rat plasma.

Methods: The ultra-performance LC-MS/MS (UPLC-MS/MS) system consisted of a Shimadzu Nexera X2 UPLC system and a 4000 QTRAP® MS/MS system. Separation was achieved with an Acquity UPLC BEH C_{18} column (2.1 x 50 mm, 1.7 µm) using 0.1% formic acid in acetonitrile and 0.1% formic acid in water as mobile phase under gradient elution with a flow rate of 0.4 mL/min. Multiple Reaction Monitoring (MRM) data were collected under positive mode to detect transitions *m/z* 325→ *m/z* 205 for OJT007, and *m/z* 350→ *m/z* 101 for Voriconazole (Internal Standard). LC-MS/MS assay validated according to Center for Drug Evaluation and Research (CDER) "Guidance for Industry: Bioanalytical Method Validation" with respect to specificity, lower limit of quantification (LLOQ), linearity and range, accuracy and precision, extraction recovery, matrix effect, carryover effect, stability.

Results: The standard curves of OJT007 in plasma were linear in the concentration range of 5-1000 ng/mL. The mean extraction recovery for OJT007 were 101.71± 5.52 at the low QC (15 ng/ml), 98.99± 3.64 at medium QC (200 ng/ml) and 95.80± 1.72 at the high QC concentration (800 ng/ml) suggesting that sample preparation resulted in a high and stable extraction recovery. The mean matrix effect for OJT007 were 8.01± 4.87, 4.43± 2.19 and 7.97± 2.52 at the low, medium, and high QC respectively, suggesting sample preparation yielded no measurable matrix effect interfering with determination of drug in biological matrix. The intra- and inter-day accuracy (% relative error, %RE) ranged from 3.19-9.78% and 5.61-10.25%, respectively. The intra- and inter-day precision (coefficient of variation, CV%) ranged from 2.77-10.25% and 2.91-7.88%, respectively.

Conclusion: A sensitive, specific, and reproducible LC-MS/MS method was developed and validated for the quantification of a novel antileishmanial agent in rat plasma. This method was validated to be linear, accurate and precise over the concentration range 5-1000 ng/ml. The method displayed good recovery without interference from endogenous components in plasma. OJT007 remains stable under the expected sample handling, storage, preparation, and analysis conditions This method will subsequently be applied for in-vitro and in-vivo studies.

Acknowledgements: This study was supported by the National Institute on Minority Health and Health Disparities of the National Institute of Health under Award Number G12MD007605.

Mitophagy-Inducing Compounds as Potential Agents for Leukemia Eradication

Panina SB, Pei J, Tolar JG, Kirienko NV
Department of BioSciences, Rice University, Houston, TX

Corresponding author: Natalia V. Kirienko, PhD, BioSciences, Rice University, 6100 Main St, Houston, TX, e-mail: kirienko@rice.edu

Background. Acute myeloid leukemia (AML) is a diverse group of hematological cancers, characterized by malignant clonal proliferation of immature myeloid progenitor cells in the bone marrow and peripheral blood. AML course is usually aggressive, and risk of relapse is >50% even for younger adults (Dohner et al. *Blood* 2010). New selective treatments are clearly essential to prolong relapse-free survival of both younger and elderly patients. Previously, we identified AML as a class of tumors that are particularly sensitive to mitochondria-targeted drugs (Panina et al. *Cell Death Dis* 2019). Furthermore, mitophagy as a selective autophagy of mitochondria has been shown to be altered in AML (Watson et al. *Cell Death Discov* 2015; Pei et al. *Cell Stem Cell* 2018) and activation of mitophagy might be a beneficial approach of leukemia eradication (Dany et al. *Blood* 2016).

Goal. To assess if novel mitophagy-inducing compounds identified in *C. elegans* model have a potential in eradicating leukemia cells.

Methods. In this study, we used mitophagy-inducing compounds (PINK-1 stabilizers, or PS) identified in *C. elegans* model (n = 8). Their cytotoxicity against AML cells (MOLM-13) and toxicity in normal blood cells (PBMCs) derived from the blood of healthy donors were determined. For each effective compound which induced at least 15% drop in viability at 10 μM under 72 h treatment, LD50 dose was computed. Using chemical structure of effective parental compounds as a basis, commercially-available analogs were purchased and analyzed similarly. After ranking based on their effectiveness, PS compounds with LD50 ≤ 5 μM in MOLM-13 cells and selectivity ratio ≥ 10 were tested in other leukemia cell lines as well as included in mechanistic studies, e.g. reactive oxygen species (ROS), ATP and MMP (mitochondrial membrane potential) measurements.

Results and conclusions. Out of 8 parental PS compounds from primary *C. elegans* screen, 6 (PS30, 34, 83, 103, 106, 127) passed the initial effectiveness criterion. Of these 6 compounds along with their 33 analogs (n = 39 in total), 12 molecules had LD50 ≤ 5 μM in MOLM-13 cells. Top 6 leads with highest effectiveness and selectivity included analogs of PS127 compound (PS127E, G, B, B1, F) and PS30B molecule. The latter did not affect viability of normal blood cells even at 100 μM at 72 h. Most effective leads were PS127B, E, G with LD50 of 200-500 nM in MOLM-13 cells. In addition, these drugs were also cytotoxic against other AML cell lines (MV-4;11, OCI-AML2, THP-1, MOLM-14), except PS30B in OCI-AML2. Mechanistically, PS127 analogs induced ROS generation in AML cells, but not PBMCs, under 24 h treatment with LD50 dose. At the same time, they did not significantly change MMP under these conditions. On other hand, PS30B did not affect ROS level, but decreased MMP in AML cells. At 16 h of treatment an estimated drop in ATP level was higher in MOLM-13 cells than PBMCs (being most profound in case of PS127B), reflecting selective effect of these compounds on leukemia mitochondrial metabolism. Finally, PS127B and E analogs exhibited strong synergetic effects specifically in AML cells when combined with Complex I inhibitor IACS-010759. This led to 2.3-4.6-fold higher level of death in AML cells. These data support the potential of these mitophagy-inducing compounds for chemical optimization and future therapeutic development.

Acknowledgments. The study was supported by the CPRIT grant RR150044, NIH NIGMS grant R35GM129294 to NVK, and NIH NIGMS grant T32GM120011 to JGT.

Utilizing Synergistic Potential of Mitochondria-Targeted Drugs for Leukemia Therapy

Pei J[1], Panina SB[1], Baran N[2], Konopleva M[2], Kirienko NV[1]

1. Department of BioSciences, Rice University, Houston, TX
2. Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX

Corresponding author: Natalia V. Kirienko, Department of BioSciences, Rice University, Houston, TX, kirienko@rice.edu

Background: Acute myeloid leukemia (AML) is an aggressive group of cancers with high mortality rates and significant relapse risks. Current treatments for AML, called induction and consolidation, exhibit poor treatment outcomes on elder patients and are insufficient against relapse problem in AML. Novel and more effective treatments are clearly necessary.

Goals: Recent discoveries suggest that AML may be particularly sensitive to chemotherapeutics that target mitochondria. Development of new combinatorial therapeutic regimens utilizing this sensitivity may resolve some limitations of single drugs and improve treatment efficacy.

Methods: To investigate the mitochondria sensitivity in AML, six compounds that target mitochondria [IACS-010759, rotenone, cytarabine, etoposide, ABT-199 (venetoclax), and carbonyl cyanide m -chlorophenylhydrazone] were each paired with six compounds with other activities, including tyrosine kinase inhibitors (midostaurin and dasatinib), glycolytic inhibitors (2-deoxy-D-glucose, 3-bromopyruvate, and lonidamine), and the microtubule destabilizer vinorelbine. The resulting combinations were tested for synergistic cytotoxicity against AML, CML (chronic myelogenous leukemia), and ALL (acute lymphoblastic leukemia) cell lines in short-term (24 h) and long-term (72h) treatments. Mitochondria function, including respiration rate, membrane potential, and ATP level in AML cell lines and primary AML samples were measured after treatment and compared with healthy blood cell control, PBMCs.

Results: 22 of 36 combinations tested showed synergistic cytotoxicity in both MOLM-13 and OCI-AML2 cell lines, with four most selective combinations being IACS-010759/vinorelbine, rotenone/2-deoxy-D-glucose, carbonyl cyanide m -chlorophenylhydrazone/dasatinib, and venetoclax/lonidiamine. In 72 h treatment, the synergistic cytotoxicity of these combinations remained, even when decreasing drugs' concentrations to nanomolar scale. The survival of healthy PBMCs was dramatically higher than AML cell lines after treatment. Among these drug pairs, IACS-010759/vinorelbine impairs several mitochondrial function and decreased ATP level most profoundly, including in primary AML samples. Some of the selected treatments were also effective in K-562, KU812 (CML) and CCRF-CEM, MOLT-4 (ALL) cell lines, and in primary AML cells from patient samples.

Conclusions: Selected drug combinations pairs were effective against AML, CML, and ALL cell lines, suggesting that these combinations may have value in treating various forms of leukemia. Some of the selected drug combinations impaired several mitochondria functions, including oxygen consumption rate (OCR) and membrane potential. Some of the selected combinations also significantly inhibited basal mitochondrial respiration in primary AML sample. Lastly, two selected combinations retained high synergy and strong selectivity in primary AML cells from patient samples, suggesting that these treatments have potential.

Acknowledgements: The study was supported by the CPRIT grant RR150044, as well as Welch Foundation grant C-1930 and NIH NIGMS grant R35GM129294 to NVK.

Novel Immune Modulators Enhance Caenorhabditis elegans Resistance to Multiple Pathogens

Revtovich AV, Hummell NA, Kirienko NV

Department of BioSciences, Rice University, Houston, TX

Corresponding author: Natalia V. Kirienko, Department of BioSciences, Rice University, Houston, TX, kirienko@rice.edu

Background. Traditionally, treatments for bacterial infection have focused on killing the microbe or preventing its growth. As antimicrobial resistance becomes more ubiquitous, the feasibility of this approach is beginning to wane and attention has begun to shift toward disrupting the host-pathogen interaction by improving the host defense.

Hypothesis. We hypothesize that small molecules that can modulate key innate immune pathways will be able to enhance host survival in the presence of pathogens.

Methods. We performed a high-throughput, high-content, phenotypic, fragment-based screen to identify small molecules that alleviate *Pseudomonas aeruginosa*-mediated killing of *Caenorhabditis elegans*. Screen hits were placed into antimicrobial, anti-virulents, or immune modulator categories based on MIC/EC ratio (EC, effective concentration, minimum amount of compound required for statistically-significant rescue; MIC, minimum inhibitory concentration, amount of the compound required to interfere with bacterial growth), and expression of *C. elegans* host defense genes. Transcriptome profiling was performed for the five selected hits. Bioinformatic, cell biology, and genetic assays were used to identify target pathways for these molecules. Compunds' ability to rescue C. elegans from Gram-positive pathogens *S. aureus* and *E. faecalis* was also tested.

Results. We identified over 20 compounds that stimulated host defense gene expression. Five of these molecules were selected for further characterization. Four of five compounds showed little toxicity against mammalian cells or worms, consistent with their identification in a phenotypic, high-content screen. Each of the compounds activated several host defense pathways, but the pathways were generally dispensable for compound-mediated rescue in Liquid Killing, suggesting redundancy or that the activation of one or more unknown pathways may be driving compound effects. A genetic mechanism was identified for LK56, which required the Mediator subunit MDT-15/MED15 and NHR-49/HNF4 for its function. Interestingly, LK32, LK34, LK38, and LK56 also rescue *C. elegans* from *P. aeruginosa* in an agar-based assay, which uses different virulence factors and defense mechanisms. Rescue in an agar-based assay for LK38 entirely depended upon the PMK-1/p38 MAPK pathway. Three compounds, LK32, LK34, and LK56 also conferred resistance to *Enterococcus faecalis*, and the two lattermost, LK34 and LK56, also reduced pathogenesis from *Staphylococcus aureus*.

Conclusions. This study supports a growing role for MDT-15 and NHR-49 in immune response. It also paves the way for future characterization of the anti-infective activity of the molecules in higher organisms and highlight the compounds' potential utility for further investigation of immune modulation as a novel therapeutic approach.

Acknowledgements: The study was supported by the Welch Foundation grant C-1930, NIH NIGMS grant R35GM129294, and John S. Dunn Foundation Collaborative Research Award Program administered by the Gulf Coast Consortia to NVK.

Development of Allosteric Modulators of the Voltage-Gated Sodium Channel 1.1

Singh AK, Tapia CM, Dvorak NM, Wadsworth PA, Wong J, Ali S, Laezza F

Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX, USA

Corresponding Author: Fernanda Laezza, Department of Pharmacology and Toxicology, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555, USA. E-mail: felaezza@utmb.edu

Background: The voltage-gated Na^+ (Na_v) channel isoforms 1.1, 1.2, and 1.6 serve as the fundamental molecular determinants of electrical signaling in the central nervous system (CNS). Crucially, these three isoforms are unevenly expressed in different neuronal subpopulations. Specifically, $Na_v1.1$ channels are abundantly expressed in inhibitory parvalbumin (PV) interneurons, whereas $Na_v1.6$ channels are abundantly expressed in excitatory pyramidal neurons of the striatum. Given the oftentimes opposing roles of $Na_v1.1$ and $Na_v1.6$ channels in regulating the excitatory/inhibitory (E/I) tone of the striatum, the development of isoform selective, allosteric modulators of $Na_v1.1$ channels could serve as a novel therapeutic strategy for the treatment of a diverse array of channelopathies.
Hypothesis/Goals: In an effort to develop small molecules capable of treating neurologic disorders conferred via perturbation of the E/I tone, a drug-discovery campaign that employed *in silico* to *ex vivo* methodologies was pursued to develop isoform selective, allosteric modulators of $Na_v1.1$ channels. In particular, the protein:protein interaction (PPI) interface between the C-terminal domain (CTD) of $Na_v1.1$ and fibroblast growth factor 14 (FGF14) was targeted, as PPI interfaces are flexible and dynamic surfaces that structurally diverge among Na_v and FGF isoform complexes.
Methods: *In silico* screening, surface plasmon resonance (SPR), and whole-cell patch-clamp electrophysiology.
Results: To begin this drug discovery campaign to develop allosteric modulators of $Na_v1.1$ by targeting its PPI interface with FGF14, a ligand-based high-throughput virtual screening of small molecules against the PPI interface was conducted using Autodock. In this virtual screening, a grid box encompassing a portion of the FENYYV sequence (residues 155-160) of FGF14 within a distance of 8Å from the Nav1.1 C-tail was targeted, a structural region of FGF14 that is predicted to be part of a druggable pocket within the FGF14:$Na_v1.1$ PPI interface. As a result of this *in silico* screening, which included 642,759 ligands, 1001 ZINC compounds were predicted to interact with this structural region of the PPI interface. Of these 1001 ZINC compounds, two lead compounds, ZINC1 and ZINC3, were identified on account of their binding scores and drug-like properties. Protein:ligand binding of these two compounds using surface plasmon resonance (SPR) confirmed that ZINC3, but not ZINC1, demonstrated appreciable binding to FGF14 at its predicted interaction site with the CTD of $Na_v1.1$. Functional evaluation of ZINC3 as a modulator of $Na_v1.1$ channel kinetics revealed that the small molecule altered $Na_v1.1$-mediated peak current density in the presence of FGF14-1b, whereas in the presence of FGF14-1a, the compound altered the $V_{1/2}$ of $Na_v1.1$ channel activation, biophysical perturbations that collectively make plausible that the compound would modulate intrinsic excitability of PV neurons of the nucleus accumbens. **Conclusions:** ZINC3 regulates biophysical properties of $Na_v1.1$ channels in a fashion that is dependent upon the presence of FGF14 isoforms expressed, illuminating its selective modulatory effects. Collectively considered, these findings suggest that ZINC3 could serve as a chemical scaffold for the optimization of neurotherapeutics to treat channelopathies conferred via perturbation of the E/I tone of the NAc.
Acknowledgements: We acknowledge our funding sources as follows: NIH Grant R01MH095995 (FL), R01MH12435101 (FL) NIH Grant R01MH111107 (FL, JZ), John Sealy Memorial Endowment Funds (FL), R01 DA038446 (JZ), P30 DA028821 (JZ), CPRIT RP150578 (CS), PhRMA Foundation (PAW), and HAMBP training grant no. T32GM008280 (NMD).

Peptide Mimicking Crucial Residues of the β12 Sheet of Fibroblast Growth Factor 14 Modulates Accumbal Excitability via Interactions with the Voltage-Gated Na^+ Channel 1.6

Singh AK,[1] Wadsworth PA,[1] Tapia CM,[1] Aceto G,[2] Ali S,[1] Chen H,[1] Dvorak N,[1] D'Ascenzo M,[2] Zhou J,[1] Laezza F[1]

1. Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX, USA
2. Department of Neuroscience, Università Cattolica del Sacro Cuore, Rome, Italy.

Corresponding Author: Fernanda Laezza, Department of Pharmacology and Toxicology, University of Texas Medical Branch, 301 University Boulevard, Galveston, TX 77555, USA. E-mail: felaezza@utmb.edu

Background: Voltage-gated Na^+ (Na_v) channels, with the support of a diverse ensemble of auxiliary proteins tightly regulating their function, serve as the fundamental molecular determinants of the initiation and propagation of action potentials in excitable cells. Foremost among auxiliary proteins that regulate the activity of $Na_v1.6$ channels is fibroblast growth factor 14 (FGF14), whose direct binding to the C-terminal domain (CTD) of $Na_v1.6$ has been shown to exert modulatory effects on the biophysical properties of these channels. As perturbation of the protein:protein interaction (PPI) between FGF14 and the CTD of $Na_v1.6$ has been shown to cause neural circuity aberrations implicated in the etiology of neurologic and neuropsychiatric disorders, this PPI interface stands out as a promising target for ion channel drug discovery campaigns.
Hypothesis/Goals: In an effort to pharmacologically modulate the PPI between FGF14 and the CTD of $Na_v1.6$, model-guided studies of the PPI interface were employed to identify peptides capable of regulating FGF14:$Na_v1.6$ complex assembly. Peptides predicted to be capable of modulating FGF14:$Na_v1.6$ complex assembly *in silico* were subsequently synthesized and tested *in vitro* to assess their effects on the biophysical properties of $Na_v1.6$ followed by *ex vivo* studies to elucidate their effects on the excitability of medium spiny neurons (MSN) in the nucleus accumbens (NAc).
Methods: *In silico* molecular modeling, split-luciferase complementation assay (LCA), surface plasmon resonance (SPR), and whole-cell patch-clamp electrophysiology.
Results: Based upon prior investigations indicating that the β12 sheet of FGF14 is a crucial structural determinant of the protein that disproportionately contributes to its Gibbs energy of protein binding to the CTD of $Na_v1.6$, the role of the FLPK motif of this structural region was investigated for its modulatory effects on the $Na_v1.6$ channel macromolecular complex. To do so, *in silico* molecular docking of the FLPK peptide was first employed, which revealed that it was predicted to interact with so-called "hot spot" residues of the FGF14:$Na_v1.6$ PPI interface. Given these *in silico* findings, the FLPK tetrapeptide was synthesized and tested *in vitro* for its modulatory effects using the LCA, which revealed that peptide inhibits FGF14:$Na_v1.6$ complex assembly at low micromolar concentrations. To investigate if FLPK conferred functionally relevant modulation of $Na_v1.6$ channels, the effects of the peptide were assessed using whole-cell patch-clamp electrophysiology in heterologous cells. Consistent with the findings of the LCA, FLPK reversed many of the modulatory effects on $Na_v1.6$-mediated currents conferred by FGF14. Most prominently, FLPK partially reversed FGF14-mediated suppression of $Na_v1.6$-mediated currents, indicating that the tetrapeptide disrupts FGF14:$Na_v1.6$ complex assembly in a functionally relevant fashion. To assess how the *in vitro* effects of FLPK on FGF14:$Na_v1.6$ complex assembly regulated excitability of MSNs of the NAc, the *ex vivo* effects of the tetrapeptide were assayed using whole-cell patch-clamp electrophysiology in acute brain slice preparations, investigations that revealed that the peptide derived from the β12 sheet of FGF14 increased accumbal excitability.
Conclusions: The FLPK tetrapeptide could serve as a chemical scaffold for the development of therapeutics targeted the FGF14:$Na_v1.6$ PPI interface.
Acknowledgements: We acknowledge our funding sources as follows: NIH Grant R01MH095995 (FL), R01MH12435101 (FL) NIH Grant R01MH111107 (FL, JZ), John Sealy Memorial Endowment Funds (FL), R01 DA038446 (JZ), P30 DA028821 (JZ), CPRIT RP150578 (CS), PhRMA Foundation (PAW), and HAMBP training grant no. T32GM008280 (NMD).

Characterizing Novel Mitochondria-Targeting Small Molecule Drugs in Caenorhabditis elegans

Tolar JG[1], Tjahjono E[1], Kirienko NV[1]

1. Department of BioSciences, Rice University

Corresponding author: Natasha Kirienko, Department of BioSciences, Rice University, 6100 Main St, Houston, TX, E-mail: kirienko@rice.edu

Background: Mitochondria are hubs for cellular energetics. Additionally, they play critical roles in other cellular homeostasis mechanisms, such as cellular signaling and proteostasis. Many common pathologies, such as neurodegeneration and metabolic disease, are driven by mitochondrial dysfunction. Enhancement of mitochondrial quality control can benefit cell heath by promoting the clearance of damaged organelles (Murphey and Hartley. *Nature Rev. Drug Discovery* 2018). Furthermore, recent results from our lab demonstrated that by inflicting mitochondrial damage (particularly in combination with glycolytic inhibition), we can selectively trigger cancer cell death (Panina et al. *Cell Death Dis* 2019). Identifying more compounds that can alter mitochondrial parameters could lead to novel therapeutic approaches.

Goal: We aim to identify and characterize novel mitochondria-targeting small molecules that could be used to improve therapies of cancers.

Methods: We screened ~50,000 small molecules to identify hits capable of activating mitochondrial autophagy (mitophagy). The screen was performed by using a *C. elegans* fluorescent reporter which visualizes the stabilization of PINK-1/PINK1 kinase. This key regulatory enzyme phosphorylates PDR-1/Parkin2, which is necessary and sufficient for targeting mitochondria for autophagic degradation. We validated hits by investigating their effects on mitochondrial morphology in *C. elegans* (mitoGFP) and distribution of autophagy components. To elucidate the mechanisms by which these novel compounds are inducing mitophagy, we measured changes in cellular and mitochondrial parameters including ATP level, ROS level, mitochondrial membrane potential, NADH/NAD^+ ratio. Additionally, we assayed the activation of a number of stress response pathways (UPR^{mt}, $MAPK^{mt}$, ESRE network, oxidative stress, and insulin signaling).

Results: We identified 8 compounds that triggered mitophagy (termed PINK1-Stabilizing (PS) compounds). These hits induced widespread fragmentation of mitochondria and autophagy machinery recruitment as assessed by BEC-1 localization (BEC-1::mRFP), indicative of autophagosome formation. We found that most compounds altered cellular ROS (elevated in PS30 PS34, PS83, PS103, PS127 and PS143; lowered in PS106; unchanged in PS135) and significantly decreased mitochondrial membrane potential. Interestingly, three PS compounds (PS34, PS127, and PS143) were potent activators of insulin signaling (inducing DAF-16 nuclear localization). Activation of insulin signaling was shown to be necessary to activate mitophagy as *daf-16(RNAi)* diminished mitophagy activation. Additionally, these compounds mildly induced oxidative stress response (as measured by *gst-4* activation). RNAi knockdown of this pathway decreased PINK-1::GFP stabilization during PS143 stress.

Conclusions: Our screening methodology effectively identified mitophagy-activating compounds. The resulting hits altered multiple cellular parameters that are closely associated with mitochondrial functions. Lastly, our data suggests that three of the compounds elicit their effect via activation of the insulin signaling pathway.

Acknowledgements: The study was supported by the CPRIT grant RR150044, NIH NIGMS grant R35GM129294 to NVK, and NIH NIGMS grant T32GM120011 to JGT.

Developing Pharmacological Probes for Interrogation of the Circuitry of the Nucleus Accumbens

Wadsworth PA,[1] Singh AK,[1] Dvorak NM,[1] Nguyen N,[2] Aceto G,[3] Troendle E,[4] Tapia CM,[1] Wang P,[1] Wadsworth A,[1] Folorunso O,[1] Chen H,[1] Powell RT,[2] Ulmschneider M,[4] Zhou J,[1] D'Ascenzo M,[3] Stephan C,[2] Laezza F[1]

1. Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX, USA.
2. Center for Translational Cancer Research, Texas A&M Health Science Center: Institute of Biosciences and Technology, Houston, TX, USA.
3. Department of Neuroscience, Università Cattolica del Sacro Cuore, Rome, Italy.
4. Department of Chemistry, King's College London, London, UK.

Corresponding author: Dr. Fernanda Laezza, Department of Pharmacology and Toxicology, University of Texas Medical Branch, 301 University Blvd, Galveston, TX, Email: felaezza@utmb.edu.

Background: Disruption of protein:protein interactions (PPI) within the voltage-gated Na^+ (Na_v) channel macromolecular complex gives rise to neural circuity aberrations that are implicated in the etiology of neuropsychiatric disorders. Bridging the gap between disruption of PPIs and depression- and anxiety-like behaviors, however, are hampered by a lack of targeted pharmacological probes.
Hypothesis/Goals: Focusing on the PPI interface between $Na_v1.6$ and its auxiliary protein fibroblast growth factor 14 (FGF14), we screened 44,480 small molecules against this surface to identify a lead compound that could be used to study how pharmacological modulation of the PPI altered neuronal excitability of medium spiny neurons (MSN) in the nucleus accumbens (NAc) and to study how these electrophysiological changes *ex vivo* caused changes in goal-directed behaviors *in vivo*.
Methods: Split-luciferase complementation assay (LCA), surface plasmon resonance (SPR), electrophysiology, *in silico* testing of blood-brain barrier (BBB) permeability, *in vivo* pharmacokinetic (PK) and behavioral testing.
Results: Primary and counter-screening of the initial pool of 44,480 small molecules identified seven compounds capable of selectively modulating FGF14:$Na_v1.6$ complex assembly with low micromolar potency. Of these seven compounds, three small molecules were shown to reduce the binding affinity of FGF14 toward the C-terminal domain (CTD) of $Na_v1.6$ using SPR. Electrophysiological evaluation of the three compounds in heterologous cells revealed that only one, 7605, exhibited a mechanism of action (MOA) that was contingent upon the presence of FGF14. Subsequent electrophysiological evaluation of the effects of 7605 in acute brain slice preparations revealed that the small molecule suppressed intrinsic firing properties and persistent Na^+ currents in MSNs of the NAc in an FGF14-dependent manner. After an *in silico* investigation suggested that the structural features of 7605 were favorable for BBB permeability, the compound underwent *in vivo* testing to assess its PK properties and effects on behavior in rats. Although the results of the behavioral assays are pending, the PK assessment of 7605 indicates that the NAc is modestly exposed to the drug upon intravenous injection in rats, making plausible that the compound will exert modulatory effects on goal-directed behaviors.
Conclusions: Through this HTS campaign, a small molecule has been identified that selectively inhibits FGF14:$Na_v1.6$ complex assembly and resultantly leads to altered mesocorticolimbic activity. *In vivo* effects of 7605 on goal-directed behaviors are currently being assessed. These collective measures will continue to illuminate how PPIs within the Na_v channel macromolecular complex modulate neural circuity that, when perturbed, is implicated in the etiology of neurologic and neuropsychiatric disorders.
Acknowledgments: We acknowledge our funding sources as follows: NIH Grant R01MH095995 (FL), R01MH12435101 (FL) NIH Grant R01MH111107 (FL, JZ), John Sealy Memorial Endowment Funds (FL), R01 DA038446 (JZ), P30 DA028821 (JZ), CPRIT RP150578 (CS), PhRMA Foundation (PAW), and HAMBP training grant no. T32GM008280 (NMD).

A UHPLC-MS/MS Method for The Quantification of JIB-04 in Rat Plasma: Development, Validation and Application to Pharmacokinetics Study

Wang Y[1], Ma J[1], Martinez ED[2,3], Liang D[1], Xie H[1*]

1. Department of Pharmaceutical and Environmental Health Sciences, College of Pharmacy and Health Sciences, Texas Southern University, Houston, TX 77004, USA
2. Department of Pharmacology, [3]Hamon Center for Therapeutic Oncology Research, UT Southwestern Medical Center, Dallas, TX 75390, USA

*Corresponding author at: Department of Pharmaceutical and Environmental Health Sciences, College of Pharmacy and Health Sciences, Texas Southern University, 3100 Cleburne Street, Houston, TX 77004, USA. Email: huan.xie@tsu.edu

Background: JIB-04 is a small molecule that pan-selectively inhibits lysine demethylases (KDMs), showing maximal inhibitory activity against KDM5A, and as secondary targets, KDM4D/4B/4A/6B/4C. Recently, it was found that JIB-04 also potently and selectively blocks HIV-1 Tat expression, transactivation, and virus replication in T cell lines via the inhibition of a new target, serine hydroxymethyltransferase 2.

Hypothesis/Goals: Pharmacokinetic characterization and an analytical method for the quantification of JIB-04 are necessary for the further development of this small molecule. The objective of this study is to develop and validate a UHPLC-MS/MS method for the quantification of JIB-04 in rat plasma, and to apply the method to the pharmacokinetic study of JIB-04 in rat plasma after intravenous (i.v.) and oral administration.

Methods: The assay was performed on a 6500+ Triple Quad LC-MS/MS System equipped with an ExionLC UHPLC unit (AB SCIEX LLC, CA, USA) and an ACE Excel 2 SuperC18 Column (50 x 2.1 mm, 2 µm, Advanced Chromatography Technologies Ltd., Aberdeen, Scotland, UK). The optimized method used binary gradient mobile phases with water (0.05% acetic acid) as mobile phase A and ACN (0.05% acetic acid) as mobile phase B. A flow rate of 0.5 mL/min was used with 5 µL of injection volume. Running time was 3 min. Multiple Reaction Monitoring (MRM) data were collected under positive mode to detect transitions m/z 309.1→ 230.1 for JIB-04, and m/z 310.1→ 231.0 for EPH8 (Internal Standard). The method was validated according to the FDA and the EMA guidelines for bioanalytical method validation.

Results: The linearity of the calibration curves was found in the range of 0.5 – 1000 ng/mL. The intra-day and inter-day accuracy (RE%) was -7.4 % to 3.7 %, and the precision (CV%) ranged from 1.9 % to 10.2 % at LLOQ (0.5 ng/mL) and QC (1, 50 and 800 ng/mL) levels. Matrix effects were ranged from 87.6 ± 1.8 % to 101.0 ± 10.5 %, and the recoveries were between 104.8 ± 1.5 % and 135.1 ± 2.7 %. Following i.v. administration, high JIB-04 concentrations (5270 ± 527.2 ng/mL at 2 min after dosing) in plasma was observed immediately after dosing followed by a rapid decrease. After 24 h, JIB-04 concentration was only 3.0 ± 1.0 ng/mL. Following oral administration, JIB-04 was quickly absorbed into blood. JIB-04 concentration reached 122.9 ± 51.0 ng/mL within 5 min, and reached the Cmax in 0.5 ~ 4 h, then rapidly decreased. Four out of six subjects showed a 2nd peak concentration, and two subjects only showed one peak concentration, which implies there is individual difference among subjects. The oral bioavailability of JIB-04 was calculated to be 44.4%.

Conclusions: A sensitive, specific, fast, and reliable UHPLC- MS/MS method for the quantification of JIB-04 in rat plasma samples was developed and fully validated according to FDA and EMA guidelines. The method has been successfully applied to pharmacokinetic studies for the quantification of JIB-04 after i.v. and oral administration in rats.

Acknowledgements: This study was funded in part by Cancer Prevention & Research Institute of Texas (CPRIT) Core Facilities Support Awards (RP180748), and the National Institute of Health's Research Centers in Minority Institutes Program (RCMI, U54MD007605) to H.X. and D.L.; as well as by the Welch Foundation (I-1878), and by the John P. Perkins, Ph.D Endowment in Biomedical Science to E.D.M. We acknowledge Dr. Jung-Mo Ahn's lab at University of Texas at Dallas for synthesizing and providing JIB-04.